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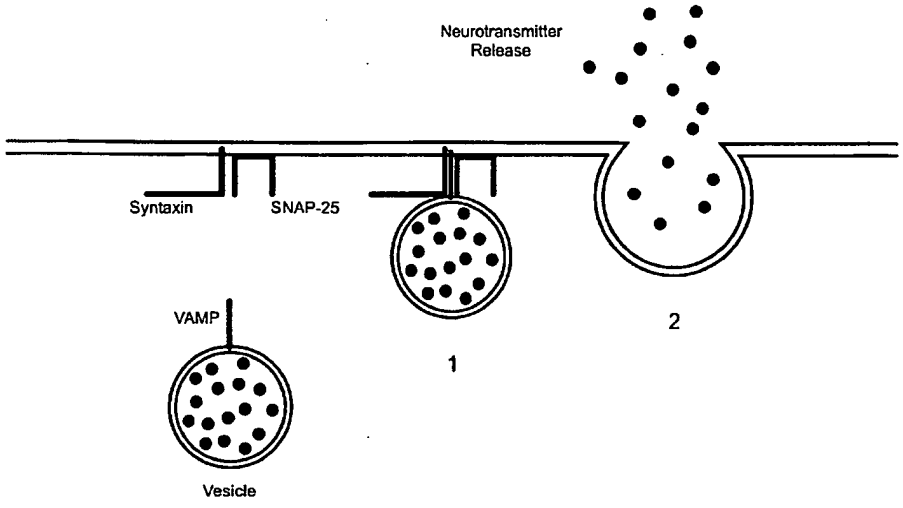
(54) Title: MODIFIED CLOSTRIDIAL TOXINS WITH ENHANCED TRANSLOCATION CAPABILITIES AND ALTERED TARGETING ACTIVITY FOR NON-CLOSTRIDIAL TOXIN TARGET CELLS



(57) Abstract: The specification discloses modified Clostridial toxins comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a translocation facilitating domain and an altered target domain; polynucleotide molecules encoding such modified Clostridial toxins; and methods of producing such modified Clostridial toxins.

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Modified Clostridial Toxins with Enhanced Translocation Capabilities and Altered Targeting Activity For Non-Clostridial Toxin Target Cells

[01] This Non-Provisional Patent Application claims priority pursuant to 35 U.S.C. §119(e) to U.S. Provisional Patent Application Serial No. 60/807,059 filed July 11, 2006, which is hereby incorporated by reference in its entirety.

[02] All patents and publications cited in this application are hereby incorporated by reference in their entirety.

[03] The ability of Clostridial toxins, such as, *e.g.*, Botulinum neurotoxins (BoNTs), BoNT/A, BoNT/B, BoNT/C1, BoNT/D, BoNT/E, BoNT/F and BoNT/G, and Tetanus neurotoxin (TeNT), to inhibit neuronal transmission are being exploited in a wide variety of therapeutic and cosmetic applications, see *e.g.*, William J. Lipham, COSMETIC AND CLINICAL APPLICATIONS OF BOTULINUM TOXIN (Slack, Inc., 2004). Clostridial toxins commercially available as pharmaceutical compositions include, BoNT/A preparations, such as, *e.g.*, BOTOX® (Allergan, Inc., Irvine, CA), Dysport®/Reloxin®, (Beaufour Ipsen, Porton Down, England), Linurase® (Prollenium, Inc., Ontario, Canada), Neuronox® (Medy-Tox, Inc., Ochang-myeon, South Korea) BTX-A (Lanzhou Institute Biological Products, China) and Xeomin® (Merz Pharmaceuticals, GmbH., Frankfurt, Germany); and BoNT/B preparations, such as, *e.g.*, MyoBloc™/NeuroBloc™ (Elan Pharmaceuticals, San Francisco, CA). As an example, BOTOX® is currently approved in one or more countries for the following indications: achalasia, adult spasticity, anal fissure, back pain, blepharospasm, bruxism, cervical dystonia, essential tremor, glabellar lines or hyperkinetic facial lines, headache, hemifacial spasm, hyperactivity of bladder, hyperhidrosis, juvenile cerebral palsy, multiple sclerosis, myoclonic disorders, nasal labial lines, spasmodic dysphonia, strabismus and VII nerve disorder.

[04] A Clostridial toxin treatment inhibits neurotransmitter release by disrupting the exocytotic process used to secret the neurotransmitter into the synaptic cleft. There is a great desire by the pharmaceutical industry to expand the use of Clostridial toxin therapies beyond its current myo-relaxant applications to treat sensory-based ailment, such as, *e.g.*, various kinds of chronic pain, as well as non-neuronal based disorders, such as, *e.g.*, pancreatitis. One approach that is currently being exploited to expand Clostridial toxin-based therapies involves modifying a Clostridial toxin so that the modified toxin has an altered cell targeting capability for a non-Clostridial toxin target cell. This re-targeted capability is achieved by replacing a naturally-occurring targeting domain of a Clostridial toxin with a targeting domain showing a selective binding activity for a non-Clostridial toxin receptor present in a non-Clostridial toxin target cell. Such modifications to a targeting domain result in a modified toxin that is able to selectively bind to a non-Clostridial toxin receptor (target receptor) present on a non-Clostridial toxin target cell (re-targeted). A modified Clostridial toxin with an altered targeting activity for a non-Clostridial toxin target cell can bind to a target receptor, translocate into the cytoplasm, and exert its proteolytic effect on the SNARE complex of the non-Clostridial toxin target cell.

[05] Non-limiting examples of modified Clostridial toxins with an altered targeting activity for a non-Clostridial toxin target cell are described in, *e.g.*, Keith A. Foster et al., *Clostridial Toxin Derivatives Able To Modify Peripheral Sensory Afferent Functions*, U.S. Patent 5,989,545 (Nov. 23, 1999); Clifford C. Shone et al., *Recombinant Toxin Fragments*, U.S. Patent 6,461,617 (Oct. 8, 2002); Conrad P. Quinn et al., *Methods and Compounds for the Treatment of Mucus Hypersecretion*, U.S. Patent 6,632,440 (Oct. 14, 2003); Lance E. Steward et al., *Methods And Compositions For The Treatment Of Pancreatitis*, U.S. Patent 6,843,998 (Jan. 18, 2005); Stephan Donovan, *Clostridial Toxin Derivatives and Methods For Treating Pain*, U.S. Patent Publication 2002/0037833 (Mar. 28, 2002); Keith A. Foster et al., *Inhibition of Secretion from Non-neural Cells*, U.S. Patent Publication 2003/0180289 (Sep. 25, 2003); J. Oliver Dolly et al., *Activatable Recombinant Neurotoxins*, WO 2001/014570 (Mar. 1, 2001); Keith A. Foster et al., *Re-targeted Toxin Conjugates*, International Patent Publication WO 2005/023309 (Mar. 17, 2005); and Lance E. Steward et al., *Multivalent Clostridial Toxin Derivatives and Methods of Their Use*, U.S. Patent Application No. 11/376,696 (Mar. 15, 2006). The ability to re-target the therapeutic effects associated with Clostridial toxins has greatly extended the number of medicinal applications able to use a Clostridial toxin therapy. As a non-limiting example, modified Clostridial toxins retargeted to sensory neurons are useful in treating various kinds of chronic pain, such as, *e.g.*, hyperalgesia and allodynia, neuropathic pain and inflammatory pain, see, *e.g.*, Foster, *supra*, (1999); and Donovan, *supra*, (2002); and Stephan Donovan, *Method For Treating Neurogenic Inflammation Pain with Botulinum Toxin and Substance P Components*, U.S. Patent 7,022,329 (Apr. 4, 2006). As another non-limiting example, modified Clostridial toxins retargeted to pancreatic cells are useful in treating pancreatitis, see, *e.g.*, Steward, *supra*, (2005).

[06] The present invention provides novel Clostridial toxins that greatly extended the number of therapeutic applications that can exploit the advantages offered by current Clostridial toxin therapies. These modified Clostridial toxins comprise, in part, a translocation facilitating domain that enhances the process by which a light chain from a modified toxin translocates into the cytoplasm of a target cell and enzymatically modify its target SNARE substrate. Thus modified Clostridial toxins with an altered targeting activity for a non-Clostridial toxin target cell, such as those disclosed in, *e.g.*, Foster, *supra*, (1999), Dolly, *supra*, (2001), Donovan, *supra*, (2002), Shone, *supra*, (2002), Foster, *supar*, (2003), Quinn, *supra*, (2003), Foster, *supra*, (2005), Steward, *supra*, (2005) and Steward, *supra*, (2006) comprising a translocation facilitating domain disclosed in the present specification, exhibit increased potency at the non-Clostridial toxin target cell. These and related advantages are useful for various clinical, therapeutic and cosmetic applications, such as, *e.g.*, the treatment of neuropathic disorders, eye disorders, pain, muscle injuries, headache, cardiovascular diseases, neuropsychiatric disorders, endocrine disorders, cancers, otic disorders, as well as, other disorders where administration of a modified Clostridial toxin with an altered targeting activity for a non-Clostridial toxin target cell to an individual can produce a beneficial effect.

BRIEF DESCRIPTION OF THE DRAWINGS

[07] FIG. 1 shows a schematic of the current paradigm of neurotransmitter release and Clostridial toxin intoxication in a central and peripheral neuron. FIG. 1A shows a schematic for the neurotransmitter release mechanism of a central and peripheral neuron. The release process can be described as comprising two steps: 1) vesicle docking, where the vesicle-bound SNARE protein of a vesicle containing neurotransmitter molecules associates with the membrane-bound SNARE proteins located at the plasma membrane; and 2) neurotransmitter release, where the vesicle fuses with the plasma membrane and the neurotransmitter molecules are exocytosed. FIG. 1B shows a schematic of the intoxication mechanism for tetanus and botulinum toxin activity in a central and peripheral neuron. This intoxication process can be described as comprising four steps: 1) receptor binding, where a Clostridial toxin binds to a Clostridial receptor and initiates the intoxication process; 2) complex internalization, where after toxin binding, a vesicle containing the toxin/receptor complex is endocytosed into the cell; 3) light chain translocation, where multiple events result in the release of the active light chain into the cytoplasm; and 4) enzymatic target modification, where the active light chain of Clostridial toxin proteolytically cleaves its target SNARE substrate, such as, *e.g.*, SNAP-25, VAMP or Syntaxin, thereby preventing vesicle docking and neurotransmitter release.

[08] FIG. 2 shows the domain organization of naturally-occurring Clostridial toxins. The single chain form depicts the amino to carboxyl linear organization comprising an enzymatic domain, a translocation domain, a H_{CN} translocation facilitating domain and a H_{CC} targeting domain. The di-chain loop region located between the translocation and enzymatic domains is depicted by the double SS bracket. This region comprises an endogenous di-chain loop protease cleavage site that upon proteolytic cleavage with a naturally-occurring protease, such as, *e.g.*, an endogenous Clostridial toxin protease or a naturally-occurring protease produced in the environment, converts the single chain form of the toxin into the di-chain form. As depicted above the single-chain form, the H_{CC} targeting domain comprises the β-trefoil domain which comprises in an amino to carboxyl linear organization of an α-fold, a β4/β5 hairpin turn, a β-fold, a β8/β9 hairpin turn and a γ-fold.

[09] FIG. 3 shows a ribbon diagram of BoNT/A illustrating the modular three-dimensional structure of the light chain (LC) comprising the enzymatic domain, the heavy chain H_N domain comprising the translocation domain, the heavy chain H_{CN} domain comprising the translocation facilitating domain and the heavy chain H_{CC} domain comprising the targeting domain.

[010] FIG. 4 shows modified Clostridial toxins with an enhanced translocation capability and an altered targeting activity located at the amino terminus of the modified toxin. FIG. 4A depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising an altered targeting domain, a translocation domain, a translocation facilitating domain and an enzymatic domain,, with the di-chain loop region depicted by the double SS bracket. A proteolytic cleavage site (P) within a di-chain loop region is located between the translocation facilitating and enzymatic domains.

Upon proteolytic cleavage with a P protease, the single chain form of the toxin is converted to the di-chain form. The P protease site can be a Clostridial toxin endogenous protease cleavage site or a non-Clostridial toxin exogenous protease cleavage site. Spacers can be placed between the targeting and translocation domains, the translocation and translocation facilitating domains, translocation facilitating and enzymatic domains or any combination thereof. FIG. 4B depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising an altered targeting domain, an enzymatic domain, a translocation domain and a translocation facilitating domain, with the di-chain loop region depicted by the double SS bracket. A proteolytic cleavage site (P) within a di-chain loop region is located between the enzymatic and translocation domains. Upon proteolytic cleavage with a P protease, the single chain form of the toxin is converted to the di-chain form. The P protease site can be a Clostridial toxin endogenous protease cleavage site or a non-Clostridial toxin exogenous protease cleavage site. Spacers can be placed between the targeting and enzymatic domains, the enzymatic and translocation domains, translocation and translocation facilitating domains or any combination thereof.

[011] FIG. 5 shows modified Clostridial toxins with an enhanced translocation capability and an altered targeting activity located between two other domains. FIG. 5A depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising an enzymatic domain, an altered targeting domain, a translocation domain and a translocation facilitating domain, with the di-chain loop region depicted by the double SS bracket. A proteolytic cleavage site (P) within a di-chain loop region is located between the enzymatic and targeting domains. Upon proteolytic cleavage with a P protease, the single chain form of the toxin is converted to the di-chain form. The P protease site can be a Clostridial toxin endogenous protease cleavage site or a non-Clostridial toxin exogenous protease cleavage site. Spacers can be placed between the enzymatic and targeting domains, the targeting and translocation domains, the translocation and translocation facilitating domains or any combination thereof. FIG. 5B depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising a translocation domain, a translocation facilitating domain, an altered targeting domain and an enzymatic domain, with the di-chain loop region depicted by the double SS bracket. A proteolytic cleavage site (P) within a di-chain loop region is located between the translocation facilitating and targeting domains. Upon proteolytic cleavage with a P protease, the single chain form of the toxin is converted to the di-chain form. The P protease site can be a Clostridial toxin endogenous protease cleavage site or a non-Clostridial toxin exogenous protease cleavage site. Spacers can be placed between the translocation and translocation facilitating domains, the translocation facilitating and targeting domains, the targeting and enzymatic domains or any combination thereof.

[012] FIG. 6 shows modified Clostridial toxins with an enhanced translocation capability and an altered targeting activity located at the carboxyl terminus of the modified toxin. FIG. 6A depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising an enzymatic domain, a translocation domain, a translocation facilitating domain and an altered targeting domain, with the di-chain loop region depicted by the double SS bracket. A proteolytic cleavage site (P)

within a di-chain loop region is located between the enzymatic and translocation domains. Upon proteolytic cleavage with a P protease, the single chain form of the toxin is converted to the di-chain form. The P protease site can be a Clostridial toxin endogenous protease cleavage site or a non-Clostridial toxin exogenous protease cleavage site. Spacers can be placed between the enzymatic and translocation domains, the translocation and translocation facilitating domains, the translocation facilitating and targeting domains or any combination thereof. FIG. 6B depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising a translocation domain, a translocation facilitating domain, an enzymatic domain and an altered targeting domain, with the di-chain loop region depicted by the double SS bracket. A proteolytic cleavage site (P) within a di-chain loop region is located between the translocation facilitating and enzymatic domains. Upon proteolytic cleavage with a P protease, the single chain form of the toxin is converted to the di-chain form. The P protease site can be a Clostridial toxin endogenous protease cleavage site or a non-Clostridial toxin exogenous protease cleavage site. Spacers can be placed between the translocation and translocation facilitating domains, the translocation facilitating and enzymatic domains, the enzymatic and targeting domains or any combination thereof.

[013] **FIG. 7** shows a ribbon diagram of BoNT/A illustrating the boundary regions of the H_{CN} domain comprising the translocation facilitating domain. FIG. 7A depicts the amino-terminal boundary region. FIG. 7B depicts the carboxyl-terminal boundary region.

DETAILED DESCRIPTION

[014] Clostridia toxins produced by *Clostridium botulinum*, *Clostridium tetani*, *Clostridium baratii* and *Clostridium butyricum* are the most widely used in therapeutic and cosmetic treatments of humans and other mammals. Strains of *C. botulinum* produce seven antigenically-distinct types of Botulinum toxins (BoNTs), which have been identified by investigating botulism outbreaks in man (BoNT/A, /B, /E and /F), animals (BoNT/C1 and /D), or isolated from soil (BoNT/G). While all seven botulinum toxins (BoNT) serotypes have similar structure and pharmacological properties, each also displays heterogeneous bacteriological characteristics. In contrast, tetanus toxin (TeNT) is produced by a uniform group of *C. tetani*. Two other species of Clostridia, *C. baratii* and *C. butyricum*, also produce toxins similar to BoNT/F and BoNT/E, respectively.

[015] Clostridia toxins possess approximately 35% amino acid identity with each other and share the same functional domain organization and overall structural architecture. Clostridial toxins are each translated as a single chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulfide loop by a naturally-occurring protease, such as, *e.g.*, an endogenous Clostridial toxin protease or a naturally-occurring protease produced in the environment (see FIG. 2). This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by a single disulfide bond and

noncovalent interactions. It is widely held that the mature di-chain molecule comprises three functionally distinct domains: 1) an enzymatic domain located in the LC that includes a metalloprotease region containing a zinc-dependent endopeptidase activity which specifically targets core components of the neurotransmitter release apparatus (Table 1); 2) a translocation domain contained within the amino-terminal half of the heavy chain (H_N domain) that facilitates release of the LC from intracellular vesicles into the cytoplasm of the target cell (Table 1); and 3) a binding domain found within the carboxyl-terminal half of the heavy chain (H_C domain) that determines the binding activity and binding specificity of the toxin to the receptor complex located at the surface of the target cell (Table 1), see, *e.g.*, Kathryn Turton et al., *Botulinum and Tetanus Neurotoxins: Structure, Function and Therapeutic Utility*, 27(11) Trends Biochem. Sci. 552-558. (2002); John A. Chaddock and P. M. H. Marks, *Clostridial Neurotoxins: Structure-Function Led Design of New Therapeutics*, 63(5) Cell. Mol. Life Sci. 540-551 (2006); and Keith Foster et al., *Re-engineering the Target Specificity of Clostridial Neurotoxins - A Route To Novel Therapeutics*, 9(2-3) Neurotox Res. 101-107 (2006).

Table 1. Clostridial Toxin Reference Sequences and Regions

Toxin	SEQ ID NO:	LC	H_N	H_C	
				H_{CN}	H_{CC}
BoNT/A	1	M1-K448	A449-I873	I874-P1110	Y1111-L1296
BoNT/B	2	M1-K441	A442-I860	L861-E1097	Y1098-E1291
BoNT/C1	3	M1-K449	T450-I868	N869-E1111	Y1112-E1291
BoNT/D	4	M1-R445	D446-I864	N865-E1098	Y1099-E1276
BoNT/E	5	M1-R422	K423-I847	K848-E1085	Y1086-K1252
BoNT/F	6	M1-K439	A440-I866	K867-K1105	Y1106-E1274
BoNT/G	7	M1-K446	S447-I865	S866-Q1105	Y1106-E1297
TeNT	8	M1-A457	S458-L881	K882-E1127	Y1128-D1315

[016] The binding, translocation and enzymatic activities of a Clostridial toxin are all necessary to execute the overall cellular intoxication mechanism whereby Clostridial toxins enter a neuron and inhibit neurotransmitter release is similar, regardless of serotype or subtype. The current paradigm describes the intoxication mechanism as comprising at least four steps: 1) receptor binding, 2) complex internalization, 3) light chain translocation, and 4) enzymatic target modification (see FIG. 1). The process is initiated when the H_C domain of a Clostridial toxin binds to a toxin-specific receptor located on the plasma membrane surface of a target cell. The binding specificity of a receptor complex is thought to be achieved, in part, by specific combinations of gangliosides and protein receptors that appear to distinctly comprise each Clostridial toxin receptor complex. Once bound, the toxin/receptor complexes are internalized by endocytosis and the internalized vesicles are sorted to specific intracellular routes. The translocation step, now thought to be mediated by the H_N domain and further facilitated by the H_{CN} domain, appears to be triggered by the acidification of the vesicle compartment. This process seems to

initiate two important pH-dependent structural rearrangements that increase hydrophobicity and promote separation of the light chain from the heavy chain of the toxin. Once activated, light chain endopeptidase of the toxin is released from the intracellular vesicle into the cytosol where it appears to specifically target one of three known core components of the neurotransmitter release apparatus. These core proteins, vesicle-associated membrane protein (VAMP)/synaptobrevin, synaptosomal-associated protein of 25 kDa (SNAP-25) and Syntaxin, are necessary for synaptic vesicle docking and fusion at the nerve terminal and constitute members of the soluble *N*-ethylmaleimide-sensitive factor-attachment protein-receptor (SNARE) family. BoNT/A and BoNT/E cleave SNAP-25 in the carboxyl-terminal region, releasing a nine or twenty-six amino acid segment, respectively, and BoNT/C1 also cleaves SNAP-25 near the carboxyl-terminus. The botulinum serotypes BoNT/B, BoNT/D, BoNT/F and BoNT/G, and tetanus toxin, act on the conserved central portion of VAMP, and release the amino-terminal portion of VAMP into the cytosol. BoNT/C1 cleaves syntaxin at a single site near the cytosolic membrane surface. The selective proteolysis of synaptic SNAREs accounts for the block of neurotransmitter release caused by Clostridial toxins *in vivo*. The SNARE protein targets of Clostridial toxins are common to exocytosis in a variety of non-neuronal types; in these cells, as in neurons, light chain peptidase activity inhibits exocytosis, see, *e.g.*, Yann Humeau et al., *How Botulinum and Tetanus Neurotoxins Block Neurotransmitter Release*, 82(5) Biochimie. 427-446 (2000); and Giovanna Lalli et al., *The Journey of Tetanus and Botulinum Neurotoxins in Neurons*, 11(9) Trends Microbiol. 431-437, (2003).

[017] The three-dimensional crystal structures of BoNT/A, BoNT/B and the H_C domain of TeNT indicate that the three functional domains of Clostridial neurotoxins are structurally distinct (see FIG. 3). The HEXXH consensus motif of the light chain forms the tetrahedral zinc binding pocket of the catalytic site located in a deep cleft on the protein surface that is accessible by a channel. The structure of the H_N and H_C domains consists primarily of β-sheet topologies that are linked by a single α-helix. The cylindrical-shaped H_N domain comprises two long amphipathic α-helices that resemble the coiled-coil motif found in some viral proteins. The H_N domain also forms a long unstructured loop called the 'translocation belt,' which wraps around a large negatively charged cleft of the light chain that blocks access of the zinc atom to the catalytic-binding pocket of active site. The H_C domain comprises two distinct structural features of roughly equal size that indicate function. The first, designated the H_{CN} domain, is located in the amino half of the H_C domain. The H_{CN} domain forms a β-barrel, jelly-roll fold. The H_{CC} domain is the second domain that comprises the H_C domain. This carboxyl-terminal domain comprises a modified β-trefoil domain which forms three distinct carbohydrate binding regions that resembles the carbohydrate binding moiety found in many sugar-binding proteins, such as, *e.g.*, serum amyloid P, sialidase, cryia, insecticidal ∂-endotoxin and lectins. Biochemical studies indicate that the β-trefoil domain structure of the H_{CC} domain appears to mediate the binding to specific carbohydrate containing components of the Clostridial toxin receptor on the cell surface, see, *e.g.*, Krzysztof Ginalski et al., *Structure-based Sequence Alignment for the Beta-Trefoil Subdomain of the Clostridial Neurotoxin Family Provides Residue Level Information About the Putative Ganglioside Binding Site*, 482(1-2) FEBS Lett. 119-124 (2000). The H_C domain tilts away from the H_N domain exposing the surface loops and making them accessible for

binding. No contacts occur between the light chain and the H_C domain.

[018] We know that only the H_{CC} domain participates in receptor binding because the β-trefoil domains are restricted to this domain. Proteins containing the structural β-trefoil domain represents a diverse group of proteins organized into at least eight superfamilies including the cytokines, MIR domain proteins, Ricin B-like lectins, agglutinins, Soybean trypsin inhibitor like proteins, Actin-crosslinking proteins, LAG-1 proteins and AbfB domain proteins, see, *e.g.*, C. A. Orengo et al., *Protein Superfamilies and Domain Superfolds*, 372 Nature 631-634 (1994); and Alexey G. Murzin et al., *SCOP: A Structural Classification of Proteins Database for the Investigation of Sequences and Structures*, 247(4) J. Mol. Biol. 536-540 (1995). While having diverse cellular roles, members of these superfamilies mechanistically function via protein-protein associations through the β-trefoil domain. Of particular interest is the fact that many of these members are specifically involved in receptor interactions, including, *e.g.*, the cytokine superfamily members Fibroblast Growth Factors (FGFs) and the Interleukin-1s (IL-1s); the Ricin B-like lectins; the agglutinins; and STI-like members the Kunitz inhibitors and Clostridium neurotoxins. That only the H_{CC} domain alone mediates the cell binding step of intoxication is further supported by the finding that mutations that disrupt the receptor binding activity of Clostridial toxins have been confined to the H_{CC} domain, see, *e.g.*, Andreas Rummel et al., *The H_{CC}-Domain of Botulinum Neurotoxins A and B Exhibits a Singular Ganglioside Binding Site Displaying Serotype Specific Carbohydrate Interaction*, 51(3) Mol. Microbiol. 631-643 (2004).

[019] Because the H_{CC} domain appears not only necessary, but sufficient for selective binding of a Clostridial toxin to its receptor, we have deduced that the primary function of the H_{CN} domain of Clostridial toxins is involved in the translocation step of the intoxication process, and not in the cell binding step, because the lack of H_{CN} domain appears to reduce intoxication efficiency. For example, a modified BoNT/A comprising a Substance P targeting domain was inefficient in intoxicating its corresponding target cells. In this modified toxin, the entire BoNT/A H_C domain, comprising both the BoNT/A H_{CC} domain and the BoNT/A H_{CN} domain, was replaced by the Substance P targeting domain. Likewise, we have determined that several other modified Clostridial toxins that have replaced the entire BoNT/A H_C domain with an exogenous targeting domain have exhibited reduced intoxication capabilities. Thus, the H_{CN} domain possess a translocation facilitating function because 1) H_{CC} domain primarily mediates the receptor binding step of the intoxication process; 2) modified Clostridial toxins lacking the H_{CN} domain exhibit a reduced ability to translocate into the cytoplasm as evident by such modified toxins exhibiting decreased proteolysis of their SNARE substrates; and 3) the LC domain mediates the enzymatic activity of the toxin. While the exact translocation facilitating mechanism of the H_{CN} domain is currently not understood, the H_{CN} domain may 1) participate in the formation of an endosomal pore; 2) mediate the insertion of the pore into a vesicle membrane; 3) assist in the delivery of LC across the endosomal membrane and/or 4) serve as a structural scaffold or spacer that facilitates the appropriate orientation a the targeting domain in relationship to the translocation domain. In this last point, the H_{CN} domain would serve to orient the translocation domain to facilitate the proper presentation of the translocation domain

for insertion into the membrane following binding of the ligand by the receptor. This novel role of teh H_{CN} domain in the translocation step is contrary to the widely accepted view that the Clostridial toxin H_{CN} domain played an integral role in the cell binding step of the intoxication process.

[020] Thus, the present invention discloses modified Clostridial toxins that exhibit 1) an enhanced translocation capability; and 2) an altered targeting capability for a naturally-occurring Clostridial toxin target cell. The enhanced translocation capability is mediated by a translocation facilitating domain comprising, *e.g.*, a H_{CN} region of Clostridial toxins. The H_{CN} domain enhances the process by which the H_N domain mediates the release of the light chain from internalized intracellular vesicles into the cytoplasm of the target cell during the translocation step. Enhanced translocation capability is obtained by including or maintaining a Clostridial toxin H_{CN} domain in a modified Clostridial toxin disclosed in the present specification. The altered targeting capability for a naturally-occurring Clostridial toxin target cell is mediated by an altered targeting domain comprising a modified H_{CC} targeting domain of Clostridial toxins. The H_{CC} domain primarily determines the binding activity and binding specificity of the toxin to the receptor complex located at the surface of the target cell. Altered targeting activity is achieved by replacing a naturally-occurring H_{CC} targeting domain of a Clostridial toxin with a binding domain for a non-Clostridial toxin receptor present on a Clostridial toxin target cell

[021] Thus modified Clostridial toxins with an enhanced translocation capability will reduce the undesirable dispersal of the toxin to areas not targeted for treatment, due to the lower dose requirement, thereby reducing or preventing the undesirable side-effects associated with diffusion of a Clostridial toxin to an unwanted location. Furthermore, an altered binding activity will provide novel Clostridial toxins that greatly extended the number of therapeutic applications that can exploit the advantages offered by current Clostridial toxin therapies.

[022] Thus, aspects of the present invention provide modified Clostridial toxins comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a translocation facilitating domain and an altered targeting domain, wherein the modified Clostridial toxin exhibits a binding activity for a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell. It is envisioned that any translocation facilitating domain capable of further facilitating the translocation step of the intoxication process where the light chain is released from intracellular vesicles into the cytoplasm of the target cell will be useful to practice aspects of the present invention, including, without limitation, a Clostridial toxin translocation facilitating domain and an enveloped virus fusogenic peptide domain. Likewise, a multitude of altered targeting domains are envisioned, including, without limitation, opioids, melanocortin peptides, galanins, granins, tachykinin peptides, cholecystokinins, Neuropeptide Y related peptides, kinin peptides, PAR peptides, corticotropin-releasing hormones, thyrotropin-releasing hormones and somatostatins. It is also envisioned that the location of the altered targeting domain in the modified Clostridial toxins of the present specification can be located at the amino terminus of the toxin, between the enzymatic and translocation domains or at the carboxyl terminus of the toxin. Thus, a modified Clostridial toxins

disclosed in the present specification can comprise an amino to carboxyl domain arrangement of, *e.g.*, an altered targeting domain, a Clostridial toxin translocation domain, a translocation facilitating domain and a Clostridial toxin enzymatic domain; an altered targeting domain, a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and a translocation facilitating domain; a Clostridial toxin enzymatic domain, an altered targeting domain, a Clostridial toxin translocation domain and a translocation facilitating domain; a Clostridial toxin translocation domain, a translocation facilitating domain, an altered targeting domain and a Clostridial toxin enzymatic domain; a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a translocation facilitating domain and an altered targeting domain; and a Clostridial toxin translocation domain, a translocation facilitating domain, a Clostridial toxin enzymatic domain and an altered targeting domain.

[023] Other aspects of the present invention provide polynucleotide molecules encoding modified Clostridial toxins comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a translocation facilitating domain and an altered targeting domain, wherein the modified Clostridial toxin exhibits a binding activity for a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell. It is envisioned that any of the modified Clostridial toxins disclosed in the present specification can be encoded by a polynucleotide molecule.

[024] Other aspects of the present invention provide methods of producing a modified Clostridial toxin disclosed in the present specification, the method comprising the step of expressing in a cell a polynucleotide molecule encoding a modified Clostridial toxin comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a translocation facilitating domain and an altered targeting domain, wherein the modified Clostridial toxin exhibits a binding activity for a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell. Other aspects of the present invention provide methods of producing a modified Clostridial toxin disclosed in the present specification, the method comprising the steps of introducing in a cell an expression construct comprising a polynucleotide molecule encoding a modified Clostridial toxin comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a translocation facilitating domain and an altered targeting domain, wherein the modified Clostridial toxin exhibits a binding activity for a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell and expressing the expression construct in the cell.

[025] Aspects of the present invention provide, in part, a modified Clostridial toxin. As used herein, the term "modified Clostridial toxin" means any polypeptide that can execute the overall cellular mechanism whereby a Clostridial toxin enters a neuron and inhibits neurotransmitter release and encompasses the binding of a Clostridial toxin to a low or high affinity receptor complex, the internalization of the toxin, the translocation of the Clostridial toxin light chain into the cytoplasm and the enzymatic modification of a Clostridial toxin substrate. A modified Clostridial toxin disclosed in the present specification is distinguished from a naturally-occurring Clostridial toxin by the fact that a modified Clostridial toxin comprises a translocation facilitating domain that enhances the process by which a light chain from a

modified toxin translocates into the cytoplasm of a target cell and a modified Clostridial toxin lacks the cell binding activity of a naturally-occurring binding domain found in a Clostridial toxin. Instead, a modified Clostridial toxin disclosed in the present specification comprises an altered targeting domain that determines the binding activity of the modified Clostridial toxin to a non-Clostridial toxin receptor located at the surface of the target cell. By definition, a naturally-occurring Clostridial toxin lacks an altered targeting domain. Examples of modified Clostridial toxin are described in, *e.g.*, Keith A. Foster et al., *Clostridial Toxin Derivatives Able To Modify Peripheral Sensory Afferent Functions*, U.S. Patent 5,989,545 (Nov. 23, 1999); Clifford C. Shone et al., *Recombinant Toxin Fragments*, U.S. Patent 6,461,617 (Oct. 8, 2002); Conrad P. Quinn et al., *Methods and Compounds for the Treatment of Mucus Hypersecretion*, U.S. Patent 6,632,440 (Oct. 14, 2003); Lance E. Steward et al., *Methods And Compositions For The Treatment Of Pancreatitis*, U.S. Patent 6,843,998 (Jan. 18, 2005); Stephan Donovan, *Clostridial Toxin Derivatives and Methods For Treating Pain*, U.S. Patent Publication 2002/0037833 (Mar. 28, 2002); Keith A. Foster et al., *Inhibition of Secretion from Non-neural Cells*, U.S. Patent Publication 2003/0180289 (Sep. 25, 2003); J. Oliver Dolly et al., *Activatable Recombinant Neurotoxins*, WO 2001/014570 (Mar. 1, 2001); Keith A. Foster et al., *Re-targeted Toxin Conjugates*, International Patent Publication WO 2005/023309 (Mar. 17, 2005); and Lance E. Steward et al., *Multivalent Clostridial Toxin Derivatives and Methods of Their Use*, U.S. Patent Application No. 11/376,696 (Mar. 15, 2006). Any of the modified Clostridial toxins described in, *e.g.*, Foster, *supra*, (1999), Dolly, *supra*, (2001), Donovan, *supra*, (2002), Shone, *supra*, (2002), Foster, *supar*, (2003), Quinn, *supra*, (2003), Foster, *supra*, (2005), Steward, *supra*, (2005) and Steward, *supra*, (2006), can be further modified to include a translocation facilitating domain as disclosed in the present specification.

[026] Aspects of the present invention provide, in part, a Clostridial toxin enzymatic domain. As used herein, the term "Clostridial toxin enzymatic domain" means any Clostridial toxin polypeptide that can execute the enzymatic target modification step of the intoxication process. Thus, a Clostridial toxin enzymatic domain specifically targets a Clostridial toxin substrate and encompasses the proteolytic cleavage of a Clostridial toxin substrate, such as, *e.g.*, SNARE proteins like a SNAP-25 substrate, a VAMP substrate and a Syntaxin substrate. Non-limiting examples of a Clostridial toxin enzymatic domain include, *e.g.*, a Clostridial toxin light chain region such as, *e.g.*, a BoNT/A light chain region, a BoNT/B light chain region, a BoNT/C1 light chain region, a BoNT/D light chain region, a BoNT/E light chain region, a BoNT/F light chain region, a BoNT/G light chain region, and a TeNT light chain region.

[027] A Clostridial toxin enzymatic domain includes, without limitation, naturally occurring Clostridial toxin light chain variants, such as, *e.g.*, Clostridial toxin light chain isoforms and Clostridial toxin light chain subtypes; non-naturally occurring Clostridial toxin light chain variants, such as, *e.g.*, conservative Clostridial toxin light chain variants, non-conservative Clostridial toxin light chain variants, Clostridial toxin light chain chimerics, active Clostridial toxin light chain fragments thereof, or any combination thereof.

[028] As used herein, the term "Clostridial toxin light chain variant," whether naturally-occurring or non-naturally-occurring, means a Clostridial toxin light chain that has at least one amino acid change from the corresponding region of the disclosed reference sequences (see Table 1) and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, all Clostridial toxin light chain variants disclosed in the present specification are capable of executing the enzymatic target modification step of the intoxication process. As non-limiting examples, a BoNT/A light chain variant comprising amino acids 1-448 of SEQ ID NO: 1 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-448 of SEQ ID NO: 1; a BoNT/B light chain variant comprising amino acids 1-441 of SEQ ID NO: 2 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-441 of SEQ ID NO: 2; a BoNT/C1 light chain variant comprising amino acids 1-449 of SEQ ID NO: 3 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-449 of SEQ ID NO: 3; a BoNT/D light chain variant comprising amino acids 1-445 of SEQ ID NO: 4 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-445 of SEQ ID NO: 4; a BoNT/E light chain variant comprising amino acids 1-422 of SEQ ID NO: 5 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-422 of SEQ ID NO: 5; a BoNT/F light chain variant comprising amino acids 1-439 of SEQ ID NO: 6 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-439 of SEQ ID NO: 6; a BoNT/G light chain variant comprising amino acids 1-446 of SEQ ID NO: 7 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-446 of SEQ ID NO: 7; and a TeNT light chain variant comprising amino acids 1-457 of SEQ ID NO: 8 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-457 of SEQ ID NO: 8.

[029] It is recognized by those of skill in the art that within each serotype of Clostridial toxin there can be naturally occurring Clostridial toxin light chain variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently four BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4, with specific light chain subtypes showing approximately 95% amino acid identity when compared to another BoNT/A light chain subtype. As used herein, the term "naturally occurring Clostridial toxin light chain variant" means any Clostridial toxin light chain produced by a naturally-occurring process, including, without limitation, Clostridial toxin light chain isoforms produced from alternatively-spliced transcripts, Clostridial toxin light chain isoforms produced by spontaneous mutation and Clostridial toxin light chain subtypes. A naturally occurring Clostridial toxin light chain variant can function in substantially the same manner as the reference Clostridial toxin light chain on which the naturally occurring Clostridial toxin light chain variant is based, and can be substituted for the reference Clostridial toxin light chain in any aspect of the present invention. A naturally occurring Clostridial toxin light chain variant may substitute one or more amino acids, two or

more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids or 100 or more amino acids from the reference Clostridial toxin light chain on which the naturally occurring Clostridial toxin light chain variant is based. A naturally occurring Clostridial toxin light chain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin light chain on which the naturally occurring Clostridial toxin light chain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin light chain on which the naturally occurring Clostridial toxin light chain variant is based.

[030] A non-limiting examples of a naturally occurring Clostridial toxin light chain variant is a Clostridial toxin light chain isoform such as, *e.g.*, a BoNT/A light chain isoform, a BoNT/B light chain isoform, a BoNT/C1 light chain isoform, a BoNT/D light chain isoform, a BoNT/E light chain isoform, a BoNT/F light chain isoform, a BoNT/G light chain isoform, and a TeNT light chain isoform. A Clostridial toxin light chain isoform can function in substantially the same manner as the reference Clostridial toxin light chain on which the Clostridial toxin light chain isoform is based, and can be substituted for the reference Clostridial toxin light chain in any aspect of the present invention.

[031] Another non-limiting examples of a naturally occurring Clostridial toxin light chain variant is a Clostridial toxin light chain subtype such as, *e.g.*, a light chain from subtype BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4; a light chain from subtype BoNT/B1, BoNT/B2, BoNT/B bivalent and BoNT/B nonproteolytic; a light chain from subtype BoNT/C1-1 and BoNT/C1-2; a light chain from subtype BoNT/E1, BoNT/E2 and BoNT/E3; and a light chain from subtype BoNT/F1, BoNT/F2, BoNT/F3 and BoNT/F4.. A Clostridial toxin light chain subtype can function in substantially the same manner as the reference Clostridial toxin light chain on which the Clostridial toxin light chain subtype is based, and can be substituted for the reference Clostridial toxin light chain in any aspect of the present invention.

[032] As used herein, the term "non-naturally occurring Clostridial toxin light chain variant" means any Clostridial toxin light chain produced with the aid of human manipulation, including, without limitation, Clostridial toxin light chains produced by genetic engineering using random mutagenesis or rational design and Clostridial toxin light chains produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin light chain variants include, *e.g.*, conservative Clostridial toxin light chain variants, non-conservative Clostridial toxin light chain variants, Clostridial toxin light chain chimeric variants and active Clostridial toxin light chain fragments.

[033] As used herein, the term "conservative Clostridial toxin light chain variant" means a Clostridial toxin light chain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference

Clostridial toxin light chain sequence (Table 1). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin light chain variant can function in substantially the same manner as the reference Clostridial toxin light chain on which the conservative Clostridial toxin light chain variant is based, and can be substituted for the reference Clostridial toxin light chain in any aspect of the present invention. A conservative Clostridial toxin light chain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the reference Clostridial toxin light chain on which the conservative Clostridial toxin light chain variant is based. A conservative Clostridial toxin light chain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin light chain on which the conservative Clostridial toxin light chain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin light chain on which the conservative Clostridial toxin light chain variant is based. Non-limiting examples of a conservative Clostridial toxin light chain variant include, *e.g.*, conservative BoNT/A light chain variants, conservative BoNT/B light chain variants, conservative BoNT/C1 light chain variants, conservative BoNT/D light chain variants, conservative BoNT/E light chain variants, conservative BoNT/F light chain variants, conservative BoNT/G light chain variants, and conservative TeNT light chain variants.

[034] As used herein, the term "non-conservative Clostridial toxin light chain variant" means a Clostridial toxin light chain in which 1) at least one amino acid is deleted from the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain variant is based; 2) at least one amino acid added to the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin light chain sequence (Table 1). A non-conservative Clostridial toxin light chain variant can function in substantially the same manner as the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain variant is based, and can be substituted for the reference Clostridial toxin light chain in any aspect of the present invention. A non-conservative Clostridial toxin light chain variant can delete one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids from the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain variant is based. A non-conservative Clostridial toxin light chain variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids to the reference Clostridial toxin light chain on which the non-conservative

Clostridial toxin light chain variant is based. A non-conservative Clostridial toxin light chain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain variant is based. A non-conservative Clostridial toxin light chain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain variant is based. Non-limiting examples of a non-conservative Clostridial toxin light chain variant include, *e.g.*, non-conservative BoNT/A light chain variants, non-conservative BoNT/B light chain variants, non-conservative BoNT/C1 light chain variants, non-conservative BoNT/D light chain variants, non-conservative BoNT/E light chain variants, non-conservative BoNT/F light chain variants, non-conservative BoNT/G light chain variants, and non-conservative TeNT light chain variants.

[035] As used herein, the term "Clostridial toxin light chain chimeric" means a polypeptide comprising at least a portion of a Clostridial toxin light chain and at least a portion of at least one other polypeptide to form a toxin light chain with at least one property different from the reference Clostridial toxin light chains of Table 1, with the proviso that this Clostridial toxin light chain chimeric is still capable of specifically targeting the core components of the neurotransmitter release apparatus and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. Such Clostridial toxin light chain chimerics are described in, *e.g.*, Lance E. Steward et al., *Leucine-based Motif and Clostridial Toxins*, U.S. Patent Publication 2003/0027752 (Feb. 6, 2003); Lance E. Steward et al., *Clostridial Neurotoxin Compositions and Modified Clostridial Neurotoxins*, U.S. Patent Publication 2003/0219462 (Nov. 27, 2003); and Lance E. Steward et al., *Clostridial Neurotoxin Compositions and Modified Clostridial Neurotoxins*, U.S. Patent Publication 2004/0220386 (Nov. 4, 2004).

[036] As used herein, the term "active Clostridial toxin light chain fragment" means any of a variety of Clostridial toxin fragments comprising the light chain can be useful in aspects of the present invention with the proviso that these light chain fragments can specifically target the core components of the neurotransmitter release apparatus and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The light chains of Clostridial toxins are approximately 420-460 amino acids in length and comprise an enzymatic domain (Table 1). Research has shown that the entire length of a Clostridial toxin light chain is not necessary for the enzymatic activity of the enzymatic domain. As a non-limiting example, the first eight amino acids of the BoNT/A light chain (residues 1-8 of SEQ ID NO: 1) are not required for enzymatic activity. As another non-limiting example,

the first eight amino acids of the TeNT light chain (residues 1-8 of SEQ ID NO: 8) are not required for enzymatic activity. Likewise, the carboxyl-terminus of the light chain is not necessary for activity. As a non-limiting example, the last 32 amino acids of the BoNT/A light chain (residues 417-448 of SEQ ID NO: 1) are not required for enzymatic activity. As another non-limiting example, the last 31 amino acids of the TeNT light chain (residues 427-457 of SEQ ID NO: 8) are not required for enzymatic activity. Thus, aspects of this embodiment can include Clostridial toxin light chains comprising an enzymatic domain having a length of, *e.g.*, at least 350 amino acids, at least 375 amino acids, at least 400 amino acids, at least 425 amino acids and at least 450 amino acids. Other aspects of this embodiment can include Clostridial toxin light chains comprising an enzymatic domain having a length of, *e.g.*, at most 350 amino acids, at most 375 amino acids, at most 400 amino acids, at most 425 amino acids and at most 450 amino acids.

[037] Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring Clostridial toxin light chain variants and non-naturally-occurring Clostridial toxin light chain variants, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[038] Global methods align sequences from the beginning to the end of the molecule and determine the best alignment by adding up scores of individual residue pairs and by imposing gap penalties. Non-limiting methods include, *e.g.*, CLUSTAL W, see, *e.g.*, Julie D. Thompson et al., *CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice*, 22(22) Nucleic Acids Research 4673-4680 (1994); and iterative refinement, see, *e.g.*, Osamu Gotoh, *Significant Improvement in Accuracy of Multiple Protein Sequence Alignments by Iterative Refinement as Assessed by Reference to Structural Alignments*, 264(4) J. Mol. Biol. 823-838 (1996).

[039] Local methods align sequences by identifying one or more conserved motifs shared by all of the input sequences. Non-limiting methods include, *e.g.*, Match-box, see, *e.g.*, Eric Depiereux and Ernest Feytmans, *Match-Box: A Fundamentally New Algorithm for the Simultaneous Alignment of Several Protein Sequences*, 8(5) CABIOS 501-509 (1992); Gibbs sampling, see, *e.g.*, C. E. Lawrence et al., *Detecting Subtle Sequence Signals: A Gibbs Sampling Strategy for Multiple Alignment*, 262(5131) Science 208-214 (1993); Align-M, see, *e.g.*, Ivo Van Walle et al., *Align-M – A New Algorithm for Multiple Alignment of Highly Divergent Sequences*, 20(9) Bioinformatics,:1428-1435 (2004).

[040] Hybrid methods combine functional aspects of both global and local alignment methods. Non-limiting methods include, *e.g.*, segment-to-segment comparison, see, *e.g.*, Burkhard Morgenstern et al., *Multiple DNA and Protein Sequence Alignment Based On Segment-To-Segment Comparison*, 93(22) Proc. Natl. Acad. Sci. U.S.A. 12098-12103 (1996); T-Coffee, see, *e.g.*, Cédric Notredame et al., *T-Coffee:*

A Novel Algorithm for Multiple Sequence Alignment, 302(1) J. Mol. Biol. 205-217 (2000); MUSCLE, see, *e.g.*, Robert C. Edgar, *MUSCLE: Multiple Sequence Alignment With High Score Accuracy and High Throughput*, 32(5) Nucleic Acids Res. 1792-1797 (2004); and DIALIGN-T, see, *e.g.*, Amarendran R Subramanian et al., *DIALIGN-T: An Improved Algorithm for Segment-Based Multiple Sequence Alignment*, 6(1) BMC Bioinformatics 66 (2005).

[041] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification comprises a Clostridial toxin enzymatic domain. In an aspect of this embodiment, a Clostridial toxin enzymatic domain comprises a naturally occurring Clostridial toxin light chain variant, such as, *e.g.*, a Clostridial toxin light chain isoform or a Clostridial toxin light chain subtype. In another aspect of this embodiment, a Clostridial toxin enzymatic domain comprises a non-naturally occurring Clostridial toxin light chain variant, such as, *e.g.*, a conservative Clostridial toxin light chain variant, a non-conservative Clostridial toxin light chain variant, a Clostridial toxin chimeric light chain, an active Clostridial toxin light chain fragment, or any combination thereof.

[042] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/A light chain. In an aspect of this embodiment, a BoNT/A light chain comprises amino acids 1-448 of SEQ ID NO: 1. In another aspect of this embodiment, a BoNT/A light chain comprises a naturally occurring BoNT/A light chain variant, such as, *e.g.*, a light chain from a BoNT/A isoform or a light chain from a BoNT/A subtype. In another aspect of this embodiment, a BoNT/A light chain comprises amino acids 1-448 of a naturally occurring BoNT/A light chain variant of SEQ ID NO: 1, such as, *e.g.*, amino acids 1-448 of a BoNT/A isoform of SEQ ID NO: 1 or amino acids 1-448 of a BoNT/A subtype of SEQ ID NO: 1. In still another aspect of this embodiment, a BoNT/A light chain comprises a non-naturally occurring BoNT/A light chain variant, such as, *e.g.*, a conservative BoNT/A light chain variant, a non-conservative BoNT/A light chain variant, a BoNT/A chimeric light chain, an active BoNT/A light chain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A light chain comprises amino acids 1-448 of a non-naturally occurring BoNT/A light chain variant of SEQ ID NO: 1, such as, *e.g.*, amino acids 1-448 of a conservative BoNT/A light chain variant of SEQ ID NO: 1, amino acids 1-448 of a non-conservative BoNT/A light chain variant of SEQ ID NO: 1, amino acids 1-448 of an active BoNT/A light chain fragment of SEQ ID NO: 1, or any combination thereof.

[043] In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at least 75% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at least 80% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at least 85% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at least 90% amino acid identity with amino acids 1-448 of SEQ ID NO: 1 or at least 95% amino acid identity with amino acids 1-448 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at most 75% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at most 80% amino acid

identity with amino acids 1-448 of SEQ ID NO: 1, at most 85% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at most 90% amino acid identity with amino acids 1-448 of SEQ ID NO: 1 or at most 95% amino acid identity with amino acids 1-448 of SEQ ID NO: 1.

[044] In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-448 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-448 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-448 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-448 of SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-448 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-448 of SEQ ID NO: 1.

[045] In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-448 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-448 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-448 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-448 of SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-448 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-448 of SEQ ID NO: 1.

[046] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/B light chain. In an aspect of this embodiment, a BoNT/B light chain comprises amino acids 1-441 of SEQ ID NO: 2. In another aspect of this embodiment, a BoNT/B light chain comprises a naturally occurring BoNT/B light chain variant, such as, *e.g.*, a light chain from a BoNT/B isoform or a light chain from a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B light chain comprises amino acids 1-441 of a naturally occurring BoNT/B light chain variant of SEQ ID NO: 2, such as, *e.g.*, amino acids 1-441 of a BoNT/B isoform of SEQ ID NO: 2 or amino acids 1-441 of a BoNT/B subtype of SEQ ID NO: 2. In still another aspect of this embodiment, a BoNT/B light chain comprises a non-naturally occurring BoNT/B light chain variant, such as, *e.g.*, a conservative BoNT/B light chain variant, a non-conservative BoNT/B light chain variant, a BoNT/B chimeric light chain, an active BoNT/B light chain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B light chain comprises amino acids 1-441 of a non-naturally occurring BoNT/B light chain variant of SEQ ID NO: 2, such as, *e.g.*, amino acids 1-441 of a conservative BoNT/B light chain variant of SEQ ID NO: 2, amino acids 1-441 of a non-conservative BoNT/B light chain variant of SEQ ID NO: 2, amino acids 1-441 of an active BoNT/B light chain fragment of SEQ ID NO: 2, or any combination thereof.

[047] In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at least 75% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at least 80% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at least 85% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at least 90% amino acid identity with amino acids 1-441 of SEQ ID NO: 2 or at least 95% amino acid identity with amino acids 1-441 of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at most 75% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at most 80% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at most 85% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at most 90% amino acid identity with amino acids 1-441 of SEQ ID NO: 2 or at most 95% amino acid identity with amino acids 1-441 of SEQ ID NO: 2.

[048] In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-441 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-441 of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-441 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-441 of SEQ ID NO: 2. In still other aspects of this

embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-441 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-441 of SEQ ID NO: 2.

[049] In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-441 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-441 of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-441 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-441 of SEQ ID NO: 2. In still other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-441 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-441 of SEQ ID NO: 2.

[050] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/C1 light chain. In an aspect of this embodiment, a BoNT/C1 light chain comprises amino acids 1-449 of SEQ ID NO: 3. In another aspect of this embodiment, a BoNT/C1 light chain comprises a naturally occurring BoNT/C1 light chain variant, such as, *e.g.*, a light chain from a BoNT/C1 isoform or a light chain from a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 light chain comprises amino acids 1-449 of a naturally occurring BoNT/C1 light chain variant of SEQ ID NO: 3, such as, *e.g.*, amino acids 1-449 of a BoNT/C1 isoform of SEQ ID NO: 3 or amino acids 1-449 of a BoNT/C1 subtype of SEQ ID NO: 3. In still another aspect of this embodiment, a BoNT/C1 light chain comprises a non-naturally occurring BoNT/C1 light chain variant, such as, *e.g.*, a conservative BoNT/C1 light chain variant, a non-conservative BoNT/C1 light chain variant, a BoNT/C1 chimeric light chain, an active BoNT/C1 light chain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 light chain comprises amino acids 1-449 of a non-naturally occurring BoNT/C1 light chain variant of SEQ ID NO: 3, such as, *e.g.*, amino acids 1-449 of a conservative BoNT/C1 light chain variant of SEQ ID NO: 3, amino acids 1-449 of a non-conservative BoNT/C1 light chain variant of SEQ ID NO: 3, amino acids 1-449 of an active BoNT/C1 light chain fragment of SEQ ID NO: 3, or any combination thereof.

[051] In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at least 75% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at least 80% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at least 85% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at least 90% amino acid identity with amino acids 1-449 of SEQ ID NO: 3 or at least 95% amino acid identity with amino acids 1-449 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at most 75% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at most 80% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at most 85% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at most 90% amino acid identity with amino acids 1-449 of SEQ ID NO: 3 or at most 95% amino acid identity with amino acids 1-449 of SEQ ID NO: 3.

[052] In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-449 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-449 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-449 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-449 of SEQ ID NO: 3. In still other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-449 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-449 of SEQ ID NO: 3.

[053] In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-449 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-449 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-449 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid

deletions relative to amino acids 1-449 of SEQ ID NO: 3. In still other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-449 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-449 of SEQ ID NO: 3.

[054] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/D light chain. In an aspect of this embodiment, a BoNT/D light chain comprises amino acids 1-445 of SEQ ID NO: 4. In another aspect of this embodiment, a BoNT/D light chain comprises a naturally occurring BoNT/D light chain variant, such as, *e.g.*, a light chain from a BoNT/D isoform or a light chain from a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D light chain comprises amino acids 1-445 of a naturally occurring BoNT/D light chain variant of SEQ ID NO: 4, such as, *e.g.*, amino acids 1-445 of a BoNT/D isoform of SEQ ID NO: 4 or amino acids 1-445 of a BoNT/D subtype of SEQ ID NO: 4. In still another aspect of this embodiment, a BoNT/D light chain comprises a non-naturally occurring BoNT/D light chain variant, such as, *e.g.*, a conservative BoNT/D light chain variant, a non-conservative BoNT/D light chain variant, a BoNT/D chimeric light chain, an active BoNT/D light chain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D light chain comprises amino acids 1-445 of a non-naturally occurring BoNT/D light chain variant of SEQ ID NO: 4, such as, *e.g.*, amino acids 1-445 of a conservative BoNT/D light chain variant of SEQ ID NO: 4, amino acids 1-445 of a non-conservative BoNT/D light chain variant of SEQ ID NO: 4, amino acids 1-445 of an active BoNT/D light chain fragment of SEQ ID NO: 4, or any combination thereof.

[055] In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at least 75% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at least 80% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at least 85% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at least 90% amino acid identity with amino acids 1-445 of SEQ ID NO: 4 or at least 95% amino acid identity with amino acids 1-445 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at most 75% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at most 80% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at most 85% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at most 90% amino acid identity with amino acids 1-445 of SEQ ID NO: 4 or at most 95% amino acid identity with amino acids 1-445 of SEQ ID NO: 4.

[056] In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-445 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four,

five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-445 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-445 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-445 of SEQ ID NO: 4. In still other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-445 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-445 of SEQ ID NO: 4.

[057] In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-445 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-445 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-445 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-445 of SEQ ID NO: 4. In still other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-445 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-445 of SEQ ID NO: 4.

[058] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/E light chain. In an aspect of this embodiment, a BoNT/E light chain comprises amino acids 1-422 of SEQ ID NO: 5. In another aspect of this embodiment, a BoNT/E light chain comprises a naturally occurring BoNT/E light chain variant, such as, *e.g.*, a light chain from a BoNT/E isoform or a light chain from a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E light chain comprises amino acids 1-422 of a naturally occurring BoNT/E light chain variant of SEQ ID NO: 5, such as, *e.g.*, amino acids 1-422 of a BoNT/E isoform of SEQ ID NO: 5 or amino acids 1-422 of a BoNT/E subtype of SEQ ID NO: 5. In still another aspect of this embodiment, a BoNT/E light chain comprises a non-naturally occurring BoNT/E light chain variant, such as, *e.g.*, a conservative BoNT/E light chain variant, a non-conservative BoNT/E light chain

variant, a BoNT/E chimeric light chain, an active BoNT/E light chain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E light chain comprises amino acids 1-422 of a non-naturally occurring BoNT/E light chain variant of SEQ ID NO: 5, such as, *e.g.*, amino acids 1-422 of a conservative BoNT/E light chain variant of SEQ ID NO: 5, amino acids 1-422 of a non-conservative BoNT/E light chain variant of SEQ ID NO: 5, amino acids 1-422 of an active BoNT/E light chain fragment of SEQ ID NO: 5, or any combination thereof.

[059] In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at least 75% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at least 80% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at least 85% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at least 90% amino acid identity with amino acids 1-422 of SEQ ID NO: 5 or at least 95% amino acid identity with amino acids 1-422 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at most 75% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at most 80% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at most 85% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at most 90% amino acid identity with amino acids 1-422 of SEQ ID NO: 5 or at most 95% amino acid identity with amino acids 1-422 of SEQ ID NO: 5.

[060] In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 5.

[061] In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 5. In other aspects of this

embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 5.

[062] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/F light chain. In an aspect of this embodiment, a BoNT/F light chain comprises amino acids 1-439 of SEQ ID NO: 6. In another aspect of this embodiment, a BoNT/F light chain comprises a naturally occurring BoNT/F light chain variant, such as, *e.g.*, a light chain from a BoNT/F isoform or a light chain from a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F light chain comprises amino acids 1-439 of a naturally occurring BoNT/F light chain variant of SEQ ID NO: 6, such as, *e.g.*, amino acids 1-439 of a BoNT/F isoform of SEQ ID NO: 6 or amino acids 1-439 of a BoNT/F subtype of SEQ ID NO: 6. In still another aspect of this embodiment, a BoNT/F light chain comprises a non-naturally occurring BoNT/F light chain variant, such as, *e.g.*, a conservative BoNT/F light chain variant, a non-conservative BoNT/F light chain variant, a BoNT/F chimeric light chain, an active BoNT/F light chain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F light chain comprises amino acids 1-439 of a non-naturally occurring BoNT/F light chain variant of SEQ ID NO: 6, such as, *e.g.*, amino acids 1-439 of a conservative BoNT/F light chain variant of SEQ ID NO: 6, amino acids 1-439 of a non-conservative BoNT/F light chain variant of SEQ ID NO: 6, amino acids 1-439 of an active BoNT/F light chain fragment of SEQ ID NO: 6, or any combination thereof.

[063] In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-439 of SEQ ID NO: 6, at least 75% amino acid identity with amino acids 1-439 of SEQ ID NO: 6, at least 80% amino acid identity with amino acids 1-439 of SEQ ID NO: 6, at least 85% amino acid identity with amino acids 1-439 of SEQ ID NO: 6, at least 90% amino acid identity with amino acids 1-439 of SEQ ID NO: 6 or at least 95% amino acid identity with amino acids 1-439 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-439 of SEQ ID NO: 6, at most 75% amino acid identity with amino acids 1-439 of SEQ ID NO: 6, at most 80% amino acid identity with amino acids 1-439 of SEQ ID NO: 6, at most 85% amino acid identity with amino acids 1-439

of SEQ ID NO: 6, at most 90% amino acid identity with amino acids 1-439 of SEQ ID NO: 6 or at most 95% amino acid identity with amino acids 1-439 of SEQ ID NO: 6.

[064] In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-439 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-439 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-439 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-439 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-439 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-439 of SEQ ID NO: 6.

[065] In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-439 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-439 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-439 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-439 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-439 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-439 of SEQ ID NO: 6.

[066] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/G light chain. In an aspect of this embodiment, a BoNT/G light chain comprises amino acids 1-446 of SEQ ID NO: 7. In

another aspect of this embodiment, a BoNT/G light chain comprises a naturally occurring BoNT/G light chain variant, such as, *e.g.*, a light chain from a BoNT/G isoform or a light chain from a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G light chain comprises amino acids 1-446 of a naturally occurring BoNT/G light chain variant of SEQ ID NO: 7, such as, *e.g.*, amino acids 1-446 of a BoNT/G isoform of SEQ ID NO: 7 or amino acids 1-446 of a BoNT/G subtype of SEQ ID NO: 7. In still another aspect of this embodiment, a BoNT/G light chain comprises a non-naturally occurring BoNT/G light chain variant, such as, *e.g.*, a conservative BoNT/G light chain variant, a non-conservative BoNT/G light chain variant, a BoNT/G chimeric light chain, an active BoNT/G light chain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G light chain comprises amino acids 1-446 of a non-naturally occurring BoNT/G light chain variant of SEQ ID NO: 7, such as, *e.g.*, amino acids 1-446 of a conservative BoNT/G light chain variant of SEQ ID NO: 7, amino acids 1-446 of a non-conservative BoNT/G light chain variant of SEQ ID NO: 7, amino acids 1-446 of an active BoNT/G light chain fragment of SEQ ID NO: 7, or any combination thereof.

[067] In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at least 75% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at least 80% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at least 85% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at least 90% amino acid identity with amino acids 1-446 of SEQ ID NO: 7 or at least 95% amino acid identity with amino acids 1-446 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at most 75% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at most 80% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at most 85% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at most 90% amino acid identity with amino acids 1-446 of SEQ ID NO: 7 or at most 95% amino acid identity with amino acids 1-446 of SEQ ID NO: 7.

[068] In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-446 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-446 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-446 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-446 of SEQ ID NO: 7. In still other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative

to amino acids 1-446 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-446 of SEQ ID NO: 7.

[069] In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-446 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-446 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-446 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-446 of SEQ ID NO: 7. In still other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-446 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-446 of SEQ ID NO: 7.

[070] In another embodiment, a Clostridial toxin enzymatic domain comprises a TeNT light chain. In an aspect of this embodiment, a TeNT light chain comprises amino acids 1-457 of SEQ ID NO: 8. In another aspect of this embodiment, a TeNT light chain comprises a naturally occurring TeNT light chain variant, such as, *e.g.*, a light chain from a TeNT isoform or a light chain from a TeNT subtype. In another aspect of this embodiment, a TeNT light chain comprises amino acids 1-457 of a naturally occurring TeNT light chain variant of SEQ ID NO: 8, such as, *e.g.*, amino acids 1-457 of a TeNT isoform of SEQ ID NO: 8 or amino acids 1-457 of a TeNT subtype of SEQ ID NO: 8. In still another aspect of this embodiment, a TeNT light chain comprises a non-naturally occurring TeNT light chain variant, such as, *e.g.*, a conservative TeNT light chain variant, a non-conservative TeNT light chain variant, a TeNT chimeric light chain, an active TeNT light chain fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT light chain comprises amino acids 1-457 of a non-naturally occurring TeNT light chain variant of SEQ ID NO: 8, such as, *e.g.*, amino acids 1-457 of a conservative TeNT light chain variant of SEQ ID NO: 8, amino acids 1-457 of a non-conservative TeNT light chain variant of SEQ ID NO: 8, amino acids 1-457 of an active TeNT light chain fragment of SEQ ID NO: 8, or any combination thereof.

[071] In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at least 75% amino acid identity

with amino acids 1-457 of SEQ ID NO: 8, at least 80% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at least 85% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at least 90% amino acid identity with amino acids 1-457 of SEQ ID NO: 8 or at least 95% amino acid identity with amino acids 1-457 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at most 75% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at most 80% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at most 85% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at most 90% amino acid identity with amino acids 1-457 of SEQ ID NO: 8 or at most 95% amino acid identity with amino acids 1-457 of SEQ ID NO: 8.

[072] In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-457 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-457 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-457 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-457 of SEQ ID NO: 8. In still other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-457 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-457 of SEQ ID NO: 8.

[073] In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-457 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-457 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-457 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-457 of SEQ ID NO: 8. In still other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20,

30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-457 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-457 of SEQ ID NO: 8.

[074] Aspects of the present invention provide, in part, a Clostridial toxin translocation domain. As used herein, the term "Clostridial toxin translocation domain" means any Clostridial toxin polypeptide that can execute the translocation step of the intoxication process that mediates Clostridial toxin light chain translocation. Thus, a Clostridial toxin translocation domain facilitates the movement of a Clostridial toxin light chain across a membrane and encompasses the movement of a Clostridial toxin light chain through the membrane an intracellular vesicle into the cytoplasm of a cell. Non-limiting examples of a Clostridial toxin translocation domain include, *e.g.*, a Clostridial toxin H_N region such as, *e.g.*, a BoNT/A H_N region, a BoNT/B H_N region, a BoNT/C1 H_N region, a BoNT/D H_N region, a BoNT/E H_N region, a BoNT/F H_N region, a BoNT/G H_N region, and a TeNT H_N region.

[075] A Clostridial toxin translocation domain includes, without limitation, naturally occurring Clostridial toxin H_N region variants, such as, *e.g.*, Clostridial toxin H_N region isoforms and Clostridial toxin H_N region subtypes; non-naturally occurring Clostridial toxin H_N region variants, such as, *e.g.*, conservative Clostridial toxin H_N region variants, non-conservative Clostridial toxin H_N region variants, Clostridial toxin H_N region chimerics, active Clostridial toxin H_N region fragments thereof, or any combination thereof.

[076] As used herein, the term "Clostridial toxin H_N region variant," whether naturally-occurring or non-naturally-occurring, means a Clostridial toxin H_N region that has at least one amino acid change from the corresponding region of the disclosed reference sequences (see Table 1) and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, all Clostridial toxin H_N region variants disclosed in the present specification are capable of executing the translocation step of the intoxication process that mediates Clostridial toxin light chain translocation. As non-limiting examples, a BoNT/A H_N region variant comprising amino acids 449-873 of SEQ ID NO: 1 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 449-873 of SEQ ID NO: 1; a BoNT/B H_N region variant comprising amino acids 442-860of SEQ ID NO: 2 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 442-860of SEQ ID NO: 2; a BoNT/C1 H_N region variant comprising amino acids 450-868 of SEQ ID NO: 3 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 450-868 of SEQ ID NO: 3; a BoNT/D H_N region variant comprising amino acids 446-864 of SEQ ID NO: 4 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 446-864 of SEQ ID NO: 4; a BoNT/E H_N region variant comprising amino acids 423-847 of SEQ ID NO: 5 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino

acid region 423-847 of SEQ ID NO: 5; a BoNT/F H_N region variant comprising amino acids 440-866 of SEQ ID NO: 6 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 440-866 of SEQ ID NO: 6; a BoNT/G H_N region variant comprising amino acids 447-865 of SEQ ID NO: 7 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 447-865 of SEQ ID NO: 7; and a TeNT H_N region variant comprising amino acids 458-881 of SEQ ID NO: 8 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 458-881 of SEQ ID NO: 8.

[077] It is recognized by those of skill in the art that within each serotype of Clostridial toxin there can be naturally occurring Clostridial toxin H_N region variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently four BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4, with specific H_N region subtypes showing approximately 87% amino acid identity when compared to another BoNT/A H_N region subtype. As used herein, the term "naturally occurring Clostridial toxin H_N region variant" means any Clostridial toxin H_N region produced by a naturally-occurring process, including, without limitation, Clostridial toxin H_N region isoforms produced from alternatively-spliced transcripts, Clostridial toxin H_N region isoforms produced by spontaneous mutation and Clostridial toxin H_N region subtypes. A naturally occurring Clostridial toxin H_N region variant can function in substantially the same manner as the reference Clostridial toxin H_N region on which the naturally occurring Clostridial toxin H_N region variant is based, and can be substituted for the reference Clostridial toxin H_N region in any aspect of the present invention. A naturally occurring Clostridial toxin H_N region variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids or 100 or more amino acids from the reference Clostridial toxin H_N region on which the naturally occurring Clostridial toxin H_N region variant is based. A naturally occurring Clostridial toxin H_N region variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin H_N region on which the naturally occurring Clostridial toxin H_N region variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin H_N region on which the naturally occurring Clostridial toxin H_N region variant is based.

[078] A non-limiting examples of a naturally occurring Clostridial toxin H_N region variant is a Clostridial toxin H_N region isoform such as, *e.g.*, a BoNT/A H_N region isoform, a BoNT/B H_N region isoform, a BoNT/C1 H_N region isoform, a BoNT/D H_N region isoform, a BoNT/E H_N region isoform, a BoNT/F H_N region isoform, a BoNT/G H_N region isoform, and a TeNT H_N region isoform. A Clostridial toxin H_N region isoform can function in substantially the same manner as the reference Clostridial toxin H_N region on

which the Clostridial toxin H_N region isoform is based, and can be substituted for the reference Clostridial toxin H_N region in any aspect of the present invention.

[079] Another non-limiting examples of a naturally occurring Clostridial toxin H_N region variant is a Clostridial toxin H_N region subtype such as, *e.g.*, a H_N region from subtype BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4; a H_N region from subtype BoNT/B1, BoNT/B2, BoNT/B bivalent and BoNT/B nonproteolytic; a H_N region from subtype BoNT/C1-1 and BoNT/C1-2; a H_N region from subtype BoNT/E1, BoNT/E2 and BoNT/E3; and a H_N region from subtype BoNT/F1, BoNT/F2, BoNT/F3 and BoNT/F4. A Clostridial toxin H_N region subtype can function in substantially the same manner as the reference Clostridial toxin H_N region on which the Clostridial toxin H_N region subtype is based, and can be substituted for the reference Clostridial toxin H_N region in any aspect of the present invention.

[080] As used herein, the term "non-naturally occurring Clostridial toxin H_N region variant" means any Clostridial toxin H_N region produced with the aid of human manipulation, including, without limitation, Clostridial toxin H_N regions produced by genetic engineering using random mutagenesis or rational design and Clostridial toxin H_N regions produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin H_N region variants include, *e.g.*, conservative Clostridial toxin H_N region variants, non-conservative Clostridial toxin H_N region variants, Clostridial toxin H_N region chimeric variants and active Clostridial toxin H_N region fragments.

[081] As used herein, the term "conservative Clostridial toxin H_N region variant" means a Clostridial toxin H_N region that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference Clostridial toxin H_N region sequence (Table 1). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin H_N region variant can function in substantially the same manner as the reference Clostridial toxin H_N region on which the conservative Clostridial toxin H_N region variant is based, and can be substituted for the reference Clostridial toxin H_N region in any aspect of the present invention. A conservative Clostridial toxin H_N region variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the reference Clostridial toxin H_N region on which the conservative Clostridial toxin H_N region variant is based. A conservative Clostridial toxin H_N region variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin H_N region on which the conservative Clostridial toxin H_N region variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95%

amino acid identity to the reference Clostridial toxin H_N region on which the conservative Clostridial toxin H_N region variant is based. Non-limiting examples of a conservative Clostridial toxin H_N region variant include, *e.g.*, conservative BoNT/A H_N region variants, conservative BoNT/B H_N region variants, conservative BoNT/C1 H_N region variants, conservative BoNT/D H_N region variants, conservative BoNT/E H_N region variants, conservative BoNT/F H_N region variants, conservative BoNT/G H_N region variants, and conservative TeNT H_N region variants.

[082] As used herein, the term "non-conservative Clostridial toxin H_N region variant" means a Clostridial toxin H_N region in which 1) at least one amino acid is deleted from the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region variant is based; 2) at least one amino acid added to the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin H_N region sequence (Table 1). A non-conservative Clostridial toxin H_N region variant can function in substantially the same manner as the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region variant is based, and can be substituted for the reference Clostridial toxin H_N region in any aspect of the present invention. A non-conservative Clostridial toxin H_N region variant can delete one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids from the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region variant is based. A non-conservative Clostridial toxin H_N region variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids to the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region variant is based. A non-conservative Clostridial toxin H_N region variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region variant is based. A non-conservative Clostridial toxin H_N region variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region variant is based. Non-limiting examples of a non-conservative Clostridial toxin H_N region variant include, *e.g.*, non-conservative BoNT/A H_N region variants, non-conservative BoNT/B H_N region variants, non-conservative BoNT/C1 H_N region variants, non-conservative BoNT/D H_N region variants, non-conservative BoNT/E H_N region variants, non-conservative

BoNT/F H_N region variants, non-conservative BoNT/G H_N region variants, and non-conservative TeNT H_N region variants.

[083] As used herein, the term "Clostridial toxin H_N region chimeric" means a polypeptide comprising at least a portion of a Clostridial toxin H_N region and at least a portion of at least one other polypeptide to form a toxin H_N region with at least one property different from the reference Clostridial toxin H_N regions of Table 1, with the proviso that this Clostridial toxin H_N region chimeric is still capable of specifically targeting the core components of the neurotransmitter release apparatus and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate.

[084] As used herein, the term "active Clostridial toxin H_N region fragment" means any of a variety of Clostridial toxin fragments comprising the H_N region can be useful in aspects of the present invention with the proviso that these active fragments can facilitate the release of the LC from intracellular vesicles into the cytoplasm of the target cell and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The H_N regions from the heavy chains of Clostridial toxins are approximately 410-430 amino acids in length and comprise a translocation domain (Table 1). Research has shown that the entire length of a H_N region from a Clostridial toxin heavy chain is not necessary for the translocating activity of the translocation domain. Thus, aspects of this embodiment can include Clostridial toxin H_N regions comprising a translocation domain having a length of, *e.g.*, at least 350 amino acids, at least 375 amino acids, at least 400 amino acids and at least 425 amino acids. Other aspects of this embodiment can include Clostridial toxin H_N regions comprising translocation domain having a length of, *e.g.*, at most 350 amino acids, at most 375 amino acids, at most 400 amino acids and at most 425 amino acids.

[085] Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring Clostridial toxin H_N region variants and non-naturally-occurring Clostridial toxin H_N region variants, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[086] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification comprises a Clostridial toxin translocation domain. In an aspect of this embodiment, a Clostridial toxin translocation domain comprises a naturally occurring Clostridial toxin H_N region variant, such as, *e.g.*, a Clostridial toxin H_N region isoform or a Clostridial toxin H_N region subtype. In another aspect of this embodiment, a Clostridial toxin translocation domain comprises a non-naturally occurring Clostridial toxin H_N region variant, such as, *e.g.*, a conservative Clostridial toxin H_N region variant, a non-conservative Clostridial toxin H_N region variant, a Clostridial toxin chimeric H_N region, an active Clostridial toxin H_N region fragment, or any combination thereof.

[087] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/A H_N region. In an aspect of this embodiment, a BoNT/A H_N region comprises amino acids 449-873 of SEQ ID NO: 1. In another aspect of this embodiment, a BoNT/A H_N region comprises a naturally occurring BoNT/A H_N region variant, such as, *e.g.*, a H_N region from a BoNT/A isoform or a H_N region from a BoNT/A subtype. In another aspect of this embodiment, a BoNT/A H_N region comprises amino acids 449-873 of a naturally occurring BoNT/A H_N region variant of SEQ ID NO: 1, such as, *e.g.*, amino acids 449-873 of a BoNT/A isoform of SEQ ID NO: 1 or amino acids 449-873 of a BoNT/A subtype of SEQ ID NO: 1. In still another aspect of this embodiment, a BoNT/A H_N region comprises a non-naturally occurring BoNT/A H_N region variant, such as, *e.g.*, a conservative BoNT/A H_N region variant, a non-conservative BoNT/A H_N region variant, a BoNT/A chimeric H_N region, an active BoNT/A H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A H_N region comprises amino acids 449-873 of a non-naturally occurring BoNT/A H_N region variant of SEQ ID NO: 1, such as, *e.g.*, amino acids 449-873 of a conservative BoNT/A H_N region variant of SEQ ID NO: 1, amino acids 449-873 of a non-conservative BoNT/A H_N region variant of SEQ ID NO: 1, amino acids 449-873 of an active BoNT/A H_N region fragment of SEQ ID NO: 1, or any combination thereof.

[088] In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 449-873 of SEQ ID NO: 1, at least 75% amino acid identity with amino acids 449-873 of SEQ ID NO: 1, at least 80% amino acid identity with amino acids 449-873 of SEQ ID NO: 1, at least 85% amino acid identity with amino acids 449-873 of SEQ ID NO: 1, at least 90% amino acid identity with amino acids 449-873 of SEQ ID NO: 1 or at least 95% amino acid identity with amino acids 449-873 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 449-873 of SEQ ID NO: 1, at most 75% amino acid identity with amino acids 449-873 of SEQ ID NO: 1, at most 80% amino acid identity with amino acids 449-873 of SEQ ID NO: 1, at most 85% amino acid identity with amino acids 449-873 of SEQ ID NO: 1, at most 90% amino acid identity with amino acids 449-873 of SEQ ID NO: 1 or at most 95% amino acid identity with amino acids 449-873 of SEQ ID NO: 1.

[089] In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 449-873 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 449-873 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 449-873 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 449-873 of SEQ ID NO: 1. In still other aspects of this

embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 449-873 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 449-873 of SEQ ID NO: 1.

[090] In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 449-873 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 449-873 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 449-873 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 449-873 of SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 449-873 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 449-873 of SEQ ID NO: 1.

[091] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/B H_N region. In an aspect of this embodiment, a BoNT/B H_N region comprises amino acids 442-860of SEQ ID NO: 2. In another aspect of this embodiment, a BoNT/B H_N region comprises a naturally occurring BoNT/B H_N region variant, such as, *e.g.*, a H_N region from a BoNT/B isoform or a H_N region from a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B H_N region comprises amino acids 442-860of a naturally occurring BoNT/B H_N region variant of SEQ ID NO: 2, such as, *e.g.*, amino acids 442-860of a BoNT/B isoform of SEQ ID NO: 2 or amino acids 442-860of a BoNT/B subtype of SEQ ID NO: 2. In still another aspect of this embodiment, a BoNT/B H_N region comprises a non-naturally occurring BoNT/B H_N region variant, such as, *e.g.*, a conservative BoNT/B H_N region variant, a non-conservative BoNT/B H_N region variant, a BoNT/B chimeric H_N region, an active BoNT/B H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B H_N region comprises amino acids 442-860of a non-naturally occurring BoNT/B H_N region variant of SEQ ID NO: 2, such as, *e.g.*, amino acids 442-860of a conservative BoNT/B H_N region variant of SEQ ID NO: 2, amino acids 442-860of a non-conservative BoNT/B H_N region variant of SEQ ID NO: 2, amino acids 442-860of an active BoNT/B H_N region fragment of SEQ ID NO: 2, or any combination thereof.

[092] In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 442-860of SEQ ID NO: 2, at least 75% amino acid identity with amino acids 442-860of SEQ ID NO: 2, at least 80% amino acid identity with amino acids 442-860of SEQ ID NO: 2, at least 85% amino acid identity with amino acids 442-860of SEQ ID NO: 2, at least 90% amino acid identity with amino acids 442-860of SEQ ID NO: 2 or at least 95% amino acid identity with amino acids 442-860of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 442-860of SEQ ID NO: 2, at most 75% amino acid identity with amino acids 442-860of SEQ ID NO: 2, at most 80% amino acid identity with amino acids 442-860of SEQ ID NO: 2, at most 85% amino acid identity with amino acids 442-860of SEQ ID NO: 2, at most 90% amino acid identity with amino acids 442-860of SEQ ID NO: 2 or at most 95% amino acid identity with amino acids 442-860of SEQ ID NO: 2.

[093] In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 442-860of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 442-860of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 442-860of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 442-860of SEQ ID NO: 2. In still other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 442-860of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 442-860of SEQ ID NO: 2.

[094] In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 442-860of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 442-860of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 442-860of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at least one, two,

three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 442-860of SEQ ID NO: 2. In still other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 442-860of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 442-860of SEQ ID NO: 2.

[095] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/C1 H_N region. In an aspect of this embodiment, a BoNT/C1 H_N region comprises amino acids 450-868 of SEQ ID NO: 3. In another aspect of this embodiment, a BoNT/C1 H_N region comprises a naturally occurring BoNT/C1 H_N region variant, such as, *e.g.*, a H_N region from a BoNT/C1 isoform or a H_N region from a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 H_N region comprises amino acids 450-868 of a naturally occurring BoNT/C1 H_N region variant of SEQ ID NO: 3, such as, *e.g.*, amino acids 450-868 of a BoNT/C1 isoform of SEQ ID NO: 3 or amino acids 450-868 of a BoNT/C1 subtype of SEQ ID NO: 3. In still another aspect of this embodiment, a BoNT/C1 H_N region comprises a non-naturally occurring BoNT/C1 H_N region variant, such as, *e.g.*, a conservative BoNT/C1 H_N region variant, a non-conservative BoNT/C1 H_N region variant, a BoNT/C1 chimeric H_N region, an active BoNT/C1 H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 H_N region comprises amino acids 450-868 of a non-naturally occurring BoNT/C1 H_N region variant of SEQ ID NO: 3, such as, *e.g.*, amino acids 450-868 of a conservative BoNT/C1 H_N region variant of SEQ ID NO: 3, amino acids 450-868 of a non-conservative BoNT/C1 H_N region variant of SEQ ID NO: 3, amino acids 450-868 of an active BoNT/C1 H_N region fragment of SEQ ID NO: 3, or any combination thereof.

[096] In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 450-868 of SEQ ID NO: 3, at least 75% amino acid identity with amino acids 450-868 of SEQ ID NO: 3, at least 80% amino acid identity with amino acids 450-868 of SEQ ID NO: 3, at least 85% amino acid identity with amino acids 450-868 of SEQ ID NO: 3, at least 90% amino acid identity with amino acids 450-868 of SEQ ID NO: 3 or at least 95% amino acid identity with amino acids 450-868 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 450-868 of SEQ ID NO: 3, at most 75% amino acid identity with amino acids 450-868 of SEQ ID NO: 3, at most 80% amino acid identity with amino acids 450-868 of SEQ ID NO: 3, at most 85% amino acid identity with amino acids 450-868 of SEQ ID NO: 3, at most 90% amino acid identity with amino acids 450-868 of SEQ ID NO: 3 or at most 95% amino acid identity with amino acids 450-868 of SEQ ID NO: 3.

[097] In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 450-868 of SEQ ID NO: 3. In other aspects of this

embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 450-868 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 450-868 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 450-868 of SEQ ID NO: 3. In still other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 450-868 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 450-868 of SEQ ID NO: 3.

[098] In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 450-868 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 450-868 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 450-868 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 450-868 of SEQ ID NO: 3. In still other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 450-868 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 450-868 of SEQ ID NO: 3.

[099] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/D H_N region. In an aspect of this embodiment, a BoNT/D H_N region comprises amino acids 446-864 of SEQ ID NO: 4. In another aspect of this embodiment, a BoNT/D H_N region comprises a naturally occurring BoNT/D H_N region variant, such as, *e.g.*, a H_N region from a BoNT/D isoform or a H_N region from a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D H_N region comprises amino acids 446-864 of a naturally occurring BoNT/D H_N region variant of SEQ ID NO: 4, such as, *e.g.*, amino acids 446-864 of a BoNT/D isoform of SEQ ID NO: 4 or amino acids 446-864 of a BoNT/D subtype of SEQ ID NO: 4. In still another

aspect of this embodiment, a BoNT/D H_N region comprises a non-naturally occurring BoNT/D H_N region variant, such as, *e.g.*, a conservative BoNT/D H_N region variant, a non-conservative BoNT/D H_N region variant, a BoNT/D chimeric H_N region, an active BoNT/D H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D H_N region comprises amino acids 446-864 of a non-naturally occurring BoNT/D H_N region variant of SEQ ID NO: 4, such as, *e.g.*, amino acids 446-864 of a conservative BoNT/D H_N region variant of SEQ ID NO: 4, amino acids 446-864 of a non-conservative BoNT/D H_N region variant of SEQ ID NO: 4, amino acids 446-864 of an active BoNT/D H_N region fragment of SEQ ID NO: 4, or any combination thereof.

[0100] In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 446-864 of SEQ ID NO: 4, at least 75% amino acid identity with amino acids 446-864 of SEQ ID NO: 4, at least 80% amino acid identity with amino acids 446-864 of SEQ ID NO: 4, at least 85% amino acid identity with amino acids 446-864 of SEQ ID NO: 4, at least 90% amino acid identity with amino acids 446-864 of SEQ ID NO: 4 or at least 95% amino acid identity with amino acids 446-864 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 446-864 of SEQ ID NO: 4, at most 75% amino acid identity with amino acids 446-864 of SEQ ID NO: 4, at most 80% amino acid identity with amino acids 446-864 of SEQ ID NO: 4, at most 85% amino acid identity with amino acids 446-864 of SEQ ID NO: 4, at most 90% amino acid identity with amino acids 446-864 of SEQ ID NO: 4 or at most 95% amino acid identity with amino acids 446-864 of SEQ ID NO: 4.

[0101] In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 446-864 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 446-864 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 446-864 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 446-864 of SEQ ID NO: 4. In still other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 446-864 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 446-864 of SEQ ID NO: 4.

[0102] In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 446-864 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 446-864 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 446-864 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 446-864 of SEQ ID NO: 4. In still other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 446-864 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 446-864 of SEQ ID NO: 4.

[0103] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/E H_N region. In an aspect of this embodiment, a BoNT/E H_N region comprises amino acids 423-847 of SEQ ID NO: 5. In another aspect of this embodiment, a BoNT/E H_N region comprises a naturally occurring BoNT/E H_N region variant, such as, *e.g.*, a H_N region from a BoNT/E isoform or a H_N region from a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E H_N region comprises amino acids 423-847 of a naturally occurring BoNT/E H_N region variant of SEQ ID NO: 5, such as, *e.g.*, amino acids 423-847 of a BoNT/E isoform of SEQ ID NO: 5 or amino acids 423-847 of a BoNT/E subtype of SEQ ID NO: 5. In still another aspect of this embodiment, a BoNT/E H_N region comprises a non-naturally occurring BoNT/E H_N region variant, such as, *e.g.*, a conservative BoNT/E H_N region variant, a non-conservative BoNT/E H_N region variant, a BoNT/E chimeric H_N region, an active BoNT/E H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E H_N region comprises amino acids 423-847 of a non-naturally occurring BoNT/E H_N region variant of SEQ ID NO: 5, such as, *e.g.*, amino acids 423-847 of a conservative BoNT/E H_N region variant of SEQ ID NO: 5, amino acids 423-847 of a non-conservative BoNT/E H_N region variant of SEQ ID NO: 5, amino acids 423-847 of an active BoNT/E H_N region fragment of SEQ ID NO: 5, or any combination thereof.

[0104] In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 423-847 of SEQ ID NO: 5, at least 75% amino acid identity with amino acids 423-847 of SEQ ID NO: 5, at least 80% amino acid identity with amino acids 423-847 of SEQ ID NO: 5, at least 85% amino acid identity with amino acids 423-847 of SEQ ID NO: 5, at least 90% amino acid identity with amino acids 423-847 of SEQ ID NO: 5 or at least 95% amino acid identity with amino acids 423-847 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E

H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 423-847 of SEQ ID NO: 5, at most 75% amino acid identity with amino acids 423-847 of SEQ ID NO: 5, at most 80% amino acid identity with amino acids 423-847 of SEQ ID NO: 5, at most 85% amino acid identity with amino acids 423-847 of SEQ ID NO: 5, at most 90% amino acid identity with amino acids 423-847 of SEQ ID NO: 5 or at most 95% amino acid identity with amino acids 423-847 of SEQ ID NO: 5.

[0105] In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 423-847 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 423-847 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 423-847 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 423-847 of SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 423-847 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 423-847 of SEQ ID NO: 5.

[0106] In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 423-847 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 423-847 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 423-847 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 423-847 of SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 423-847 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at

least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 423-847 of SEQ ID NO: 5.

[0107] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/F H_N region. In an aspect of this embodiment, a BoNT/F H_N region comprises amino acids 440-866 of SEQ ID NO: 6. In another aspect of this embodiment, a BoNT/F H_N region comprises a naturally occurring BoNT/F H_N region variant, such as, *e.g.*, a H_N region from a BoNT/F isoform or a H_N region from a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F H_N region comprises amino acids 440-866 of a naturally occurring BoNT/F H_N region variant of SEQ ID NO: 6, such as, *e.g.*, amino acids 440-866 of a BoNT/F isoform of SEQ ID NO: 6 or amino acids 440-866 of a BoNT/F subtype of SEQ ID NO: 6. In still another aspect of this embodiment, a BoNT/F H_N region comprises a non-naturally occurring BoNT/F H_N region variant, such as, *e.g.*, a conservative BoNT/F H_N region variant, a non-conservative BoNT/F H_N region variant, a BoNT/F chimeric H_N region, an active BoNT/F H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F H_N region comprises amino acids 440-866 of a non-naturally occurring BoNT/F H_N region variant of SEQ ID NO: 6, such as, *e.g.*, amino acids 440-866 of a conservative BoNT/F H_N region variant of SEQ ID NO: 6, amino acids 440-866 of a non-conservative BoNT/F H_N region variant of SEQ ID NO: 6, amino acids 440-866 of an active BoNT/F H_N region fragment of SEQ ID NO: 6, or any combination thereof.

[0108] In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 440-866 of SEQ ID NO: 6, at least 75% amino acid identity with amino acids 440-866 of SEQ ID NO: 6, at least 80% amino acid identity with amino acids 440-866 of SEQ ID NO: 6, at least 85% amino acid identity with amino acids 440-866 of SEQ ID NO: 6, at least 90% amino acid identity with amino acids 440-866 of SEQ ID NO: 6 or at least 95% amino acid identity with amino acids 440-866 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 440-866 of SEQ ID NO: 6, at most 75% amino acid identity with amino acids 440-866 of SEQ ID NO: 6, at most 80% amino acid identity with amino acids 440-866 of SEQ ID NO: 6, at most 85% amino acid identity with amino acids 440-866 of SEQ ID NO: 6, at most 90% amino acid identity with amino acids 440-866 of SEQ ID NO: 6 or at most 95% amino acid identity with amino acids 440-866 of SEQ ID NO: 6.

[0109] In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 440-866 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 440-866 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 440-866 of SEQ ID

NO: 6. In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 440-866 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 440-866 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 440-866 of SEQ ID NO: 6.

[0110] In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 440-866 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 440-866 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 440-866 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 440-866 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 440-866 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 440-866 of SEQ ID NO: 6.

[0111] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/G H_N region. In an aspect of this embodiment, a BoNT/G H_N region comprises amino acids 447-865 of SEQ ID NO: 7. In another aspect of this embodiment, a BoNT/G H_N region comprises a naturally occurring BoNT/G H_N region variant, such as, *e.g.*, a H_N region from a BoNT/G isoform or a H_N region from a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G H_N region comprises amino acids 447-865 of a naturally occurring BoNT/G H_N region variant of SEQ ID NO: 7, such as, *e.g.*, amino acids 447-865 of a BoNT/G isoform of SEQ ID NO: 7 or amino acids 447-865 of a BoNT/G subtype of SEQ ID NO: 7. In still another aspect of this embodiment, a BoNT/G H_N region comprises a non-naturally occurring BoNT/G H_N region variant, such as, *e.g.*, a conservative BoNT/G H_N region variant, a non-conservative BoNT/G H_N region variant, a BoNT/G chimeric H_N region, an active BoNT/G H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G H_N region comprises amino acids 447-865 of a non-naturally occurring BoNT/G H_N region variant of SEQ ID NO: 7, such as, *e.g.*, amino acids 447-865 of a

conservative BoNT/G H_N region variant of SEQ ID NO: 7, amino acids 447-865 of a non-conservative BoNT/G H_N region variant of SEQ ID NO: 7, amino acids 447-865 of an active BoNT/G H_N region fragment of SEQ ID NO: 7, or any combination thereof.

[0112] In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 447-865 of SEQ ID NO: 7, at least 75% amino acid identity with amino acids 447-865 of SEQ ID NO: 7, at least 80% amino acid identity with amino acids 447-865 of SEQ ID NO: 7, at least 85% amino acid identity with amino acids 447-865 of SEQ ID NO: 7, at least 90% amino acid identity with amino acids 447-865 of SEQ ID NO: 7 or at least 95% amino acid identity with amino acids 447-865 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 447-865 of SEQ ID NO: 7, at most 75% amino acid identity with amino acids 447-865 of SEQ ID NO: 7, at most 80% amino acid identity with amino acids 447-865 of SEQ ID NO: 7, at most 85% amino acid identity with amino acids 447-865 of SEQ ID NO: 7, at most 90% amino acid identity with amino acids 447-865 of SEQ ID NO: 7 or at most 95% amino acid identity with amino acids 447-865 of SEQ ID NO: 7.

[0113] In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 447-865 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 447-865 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 447-865 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 447-865 of SEQ ID NO: 7. In still other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 447-865 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 447-865 of SEQ ID NO: 7.

[0114] In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 447-865 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to

amino acids 447-865 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 447-865 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 447-865 of SEQ ID NO: 7. In still other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 447-865 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 447-865 of SEQ ID NO: 7.

[0115] In another embodiment, a Clostridial toxin translocation domain comprises a TeNT H_N region. In an aspect of this embodiment, a TeNT H_N region comprises amino acids 458-881 of SEQ ID NO: 8. In another aspect of this embodiment, a TeNT H_N region comprises a naturally occurring TeNT H_N region variant, such as, *e.g.*, a H_N region from a TeNT isoform or a H_N region from a TeNT subtype. In another aspect of this embodiment, a TeNT H_N region comprises amino acids 458-881 of a naturally occurring TeNT H_N region variant of SEQ ID NO: 8, such as, *e.g.*, amino acids 458-881 of a TeNT isoform of SEQ ID NO: 8 or amino acids 458-881 of a TeNT subtype of SEQ ID NO: 8. In still another aspect of this embodiment, a TeNT H_N region comprises a non-naturally occurring TeNT H_N region variant, such as, *e.g.*, a conservative TeNT H_N region variant, a non-conservative TeNT H_N region variant, a TeNT chimeric H_N region, an active TeNT H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT H_N region comprises amino acids 458-881 of a non-naturally occurring TeNT H_N region variant of SEQ ID NO: 8, such as, *e.g.*, amino acids 458-881 of a conservative TeNT H_N region variant of SEQ ID NO: 8, amino acids 458-881 of a non-conservative TeNT H_N region variant of SEQ ID NO: 8, amino acids 458-881 of an active TeNT H_N region fragment of SEQ ID NO: 8, or any combination thereof.

[0116] In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 458-881 of SEQ ID NO: 8, at least 75% amino acid identity with amino acids 458-881 of SEQ ID NO: 8, at least 80% amino acid identity with amino acids 458-881 of SEQ ID NO: 8, at least 85% amino acid identity with amino acids 458-881 of SEQ ID NO: 8, at least 90% amino acid identity with amino acids 458-881 of SEQ ID NO: 8 or at least 95% amino acid identity with amino acids 458-881 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 458-881 of SEQ ID NO: 8, at most 75% amino acid identity with amino acids 458-881 of SEQ ID NO: 8, at most 80% amino acid identity with amino acids 458-881 of SEQ ID NO: 8, at most 85% amino acid identity with amino acids 458-881 of SEQ ID NO: 8, at most 90% amino acid identity with amino acids 458-881 of SEQ ID NO: 8 or at most 95% amino acid identity with amino acids 458-881 of SEQ ID NO: 8.

[0117] In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 458-881 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 458-881 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 458-881 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 458-881 of SEQ ID NO: 8. In still other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 458-881 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 458-881 of SEQ ID NO: 8.

[0118] In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 458-881 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 458-881 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 458-881 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to amino acids 458-881 of SEQ ID NO: 8. In still other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 458-881 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to amino acids 458-881 of SEQ ID NO: 8.

[0119] Aspects of the present invention provide, in part, a translocation facilitating domain. As used herein, the term "translocation facilitating domain" means any polypeptide that can further facilitate the translocation step of the intoxication process that mediates Clostridial toxin light chain translocation.

Thus, a translocation facilitating domain assists the Clostridial toxin translocation domain in the movement of a Clostridial toxin light chain across a membrane and encompasses the movement of a Clostridial toxin light chain through the membrane of an intracellular vesicle into the cytoplasm of a cell. A non-limiting example of a translocation facilitating domain is a Clostridial toxin translocation facilitating domain, such as, *e.g.*, a Clostridial toxin H_{CN} region such as, *e.g.*, a BoNT/A H_{CN} region, a BoNT/B H_{CN} region, a BoNT/C1 H_{CN} region, a BoNT/D H_{CN} region, a BoNT/E H_{CN} region, a BoNT/F H_{CN} region, a BoNT/G H_{CN} region, and a TeNT H_{CN} region. Another non-limiting example of a translocation facilitating domain is a viral fusogenic peptide domain found in an enveloped virus, such as, *e.g.*, an influenzavirus, an alphavirus, a vesiculovirus, a respirovirus, a morbillivirus, an avulavirus, a henipavirus, a metapneumovirus and a foamy virus.

[0120] Thus, in an embodiment, a translocation facilitating domain assists the Clostridial toxin translocation domain in the movement of a Clostridial toxin light chain across a membrane. In aspects of this embodiment, a translocation facilitating domain assists the Clostridial toxin translocation domain in the movement of a Clostridial toxin light chain across a membrane by increasing the amount of Clostridial toxin light chain in the cytoplasm by, *e.g.*, at least 10 %, at least 20 %, at least 30 %, at least 40 %, at least 50 %, at least 60 %, at least 70 %, at least 80 %, at least 90 % or at least 100 %. In other aspects of this embodiment, a translocation facilitating domain assists the Clostridial toxin translocation domain in the movement of a Clostridial toxin light chain across a membrane by increasing the amount of Clostridial toxin light chain in the cytoplasm by, *e.g.*, at least two-fold, at least three-fold, at least four-fold, at least five-fold, at least ten-fold or at least twenty-fold. In yet other aspects of this embodiment, a translocation facilitating domain assists the Clostridial toxin translocation domain in the movement of a Clostridial toxin light chain across a membrane by increasing the amount of Clostridial toxin light chain in the cytoplasm by, *e.g.*, at most 10 %, at most 20 %, at most 30 %, at most 40 %, at most 50 %, at most 60 %, at most 70 %, at most 80 %, at most 90 % or at most 100 %. In other aspects of this embodiment, a translocation facilitating domain assists the Clostridial toxin translocation domain in the movement of a Clostridial toxin light chain across a membrane by increasing the amount of Clostridial toxin light chain in the cytoplasm by, *e.g.*, at most two-fold, at most three-fold, at most four-fold, at most five-fold, at most ten-fold or at most twenty-fold.

[0121] A Clostridial toxin translocation facilitating domain includes, without limitation, naturally occurring Clostridial toxin H_{CN} region variants, such as, *e.g.*, Clostridial toxin H_{CN} region isoforms and Clostridial toxin H_{CN} region subtypes; non-naturally occurring Clostridial toxin H_{CN} region variants, such as, *e.g.*, conservative Clostridial toxin H_{CN} region variants, non-conservative Clostridial toxin H_{CN} region variants, Clostridial toxin H_{CN} region chimerics, active Clostridial toxin H_{CN} region fragments thereof, or any combination thereof.

[0122] As used herein, the term "Clostridial toxin H_{CN} region variant," whether naturally-occurring or non-naturally-occurring, means a Clostridial toxin H_{CN} region that has at least one amino acid change from the

corresponding region of the disclosed reference sequences (see Table 1) and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, all Clostridial toxin H_{CN} region variants disclosed in the present specification are capable of further facilitating the translocation step of the intoxication process that mediates Clostridial toxin light chain translocation. As non-limiting examples, a BoNT/A H_{CN} region variant comprising amino acids 874-1110 of SEQ ID NO: 1 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 874-1110 of SEQ ID NO: 1; a BoNT/B H_{CN} region variant comprising amino acids 861-1097of SEQ ID NO: 2 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 861-1097of SEQ ID NO: 2; a BoNT/C1 H_{CN} region variant comprising amino acids 869-1111 of SEQ ID NO: 3 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 869-1111 of SEQ ID NO: 3; a BoNT/D H_{CN} region variant comprising amino acids 865-1098 of SEQ ID NO: 4 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 865-1098 of SEQ ID NO: 4; a BoNT/E H_{CN} region variant comprising amino acids 848-1085 of SEQ ID NO: 5 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 848-1085 of SEQ ID NO: 5; a BoNT/F H_{CN} region variant comprising amino acids 867-1105 of SEQ ID NO: 6 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 867-1105 of SEQ ID NO: 6; a BoNT/G H_{CN} region variant comprising amino acids 866-1105 of SEQ ID NO: 7 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 866-1105 of SEQ ID NO: 7; and a TeNT H_{CN} region variant comprising amino acids 882-1127 of SEQ ID NO: 8 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 882-1127 of SEQ ID NO: 8.

[0123] It is recognized by those of skill in the art that within each serotype of Clostridial toxin there can be naturally occurring Clostridial toxin H_{CN} region variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently four BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4, with specific H_{CN} region subtypes showing approximately 87% amino acid identity when compared to another BoNT/A H_{CN} region subtype. As used herein, the term "naturally occurring Clostridial toxin H_{CN} region variant" means any Clostridial toxin H_{CN} region produced by a naturally-occurring process, including, without limitation, Clostridial toxin H_{CN} region isoforms produced from alternatively-spliced transcripts, Clostridial toxin H_{CN} region isoforms produced by spontaneous mutation and Clostridial toxin H_{CN} region subtypes. A naturally occurring Clostridial toxin H_{CN} region variant can function in substantially the same manner as the reference Clostridial toxin H_{CN} region on which the naturally occurring Clostridial toxin H_{CN} region variant is based, and can be substituted for the reference Clostridial toxin H_{CN} region in any aspect of the present invention. A naturally occurring Clostridial toxin H_{CN} region variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino

acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids or 50 or more amino acids from the reference Clostridial toxin H_{CN} region on which the naturally occurring Clostridial toxin H_{CN} region variant is based. A naturally occurring Clostridial toxin H_{CN} region variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin H_{CN} region on which the naturally occurring Clostridial toxin H_{CN} region variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin H_{CN} region on which the naturally occurring Clostridial toxin H_{CN} region variant is based.

[0124] A non-limiting examples of a naturally occurring Clostridial toxin H_{CN} region variant is a Clostridial toxin H_{CN} region isoform such as, *e.g.*, a BoNT/A H_{CN} region isoform, a BoNT/B H_{CN} region isoform, a BoNT/C1 H_{CN} region isoform, a BoNT/D H_{CN} region isoform, a BoNT/E H_{CN} region isoform, a BoNT/F H_{CN} region isoform, a BoNT/G H_{CN} region isoform, and a TeNT H_{CN} region isoform. A Clostridial toxin H_{CN} region isoform can function in substantially the same manner as the reference Clostridial toxin H_{CN} region on which the Clostridial toxin H_{CN} region isoform is based, and can be substituted for the reference Clostridial toxin H_{CN} region in any aspect of the present invention.

[0125] Another non-limiting examples of a naturally occurring Clostridial toxin H_{CN} region variant is a Clostridial toxin H_{CN} region subtype such as, *e.g.*, a H_{CN} region from subtype BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4; a H_{CN} region from subtype BoNT/B1, BoNT/B2, BoNT/B bivalent and BoNT/B nonproteolytic; a H_{CN} region from subtype BoNT/C1-1 and BoNT/C1-2; a H_{CN} region from subtype BoNT/E1, BoNT/E2 and BoNT/E3; and a H_{CN} region from subtype BoNT/F1, BoNT/F2, BoNT/F3 and BoNT/F4. A Clostridial toxin H_{CN} region subtype can function in substantially the same manner as the reference Clostridial toxin H_{CN} region on which the Clostridial toxin H_{CN} region subtype is based, and can be substituted for the reference Clostridial toxin H_{CN} region in any aspect of the present invention.

[0126] As used herein, the term "non-naturally occurring Clostridial toxin H_{CN} region variant" means any Clostridial toxin H_{CN} region produced with the aid of human manipulation, including, without limitation, Clostridial toxin H_{CN} regions produced by genetic engineering using random mutagenesis or rational design and Clostridial toxin H_{CN} regions produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin H_{CN} region variants include, *e.g.*, conservative Clostridial toxin H_{CN} region variants, non-conservative Clostridial toxin H_{CN} region variants, Clostridial toxin H_{CN} region chimeric variants and active Clostridial toxin H_{CN} region fragments.

[0127] As used herein, the term "conservative Clostridial toxin H_{CN} region variant" means a Clostridial toxin H_{CN} region that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference Clostridial toxin H_{CN} region sequence (Table 1). Examples of properties include, without limitation, similar

size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin H_{CN} region variant can function in substantially the same manner as the reference Clostridial toxin H_{CN} region on which the conservative Clostridial toxin H_{CN} region variant is based, and can be substituted for the reference Clostridial toxin H_{CN} region in any aspect of the present invention. A conservative Clostridial toxin H_{CN} region variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids or 100 or more amino acids from the reference Clostridial toxin H_{CN} region on which the conservative Clostridial toxin H_{CN} region variant is based. A conservative Clostridial toxin H_{CN} region variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin H_{CN} region on which the conservative Clostridial toxin H_{CN} region variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin H_{CN} region on which the conservative Clostridial toxin H_{CN} region variant is based. Non-limiting examples of a conservative Clostridial toxin H_{CN} region variant include, *e.g.*, conservative BoNT/A H_{CN} region variants, conservative BoNT/B H_{CN} region variants, conservative BoNT/C1 H_{CN} region variants, conservative BoNT/D H_{CN} region variants, conservative BoNT/E H_{CN} region variants, conservative BoNT/F H_{CN} region variants, conservative BoNT/G H_{CN} region variants, and conservative TeNT H_{CN} region variants.

[0128] As used herein, the term "non-conservative Clostridial toxin H_{CN} region variant" means a Clostridial toxin H_{CN} region in which 1) at least one amino acid is deleted from the reference Clostridial toxin H_{CN} region on which the non-conservative Clostridial toxin H_{CN} region variant is based; 2) at least one amino acid added to the reference Clostridial toxin H_{CN} region on which the non-conservative Clostridial toxin H_{CN} region is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin H_{CN} region sequence (Table 1). A non-conservative Clostridial toxin H_{CN} region variant can function in substantially the same manner as the reference Clostridial toxin H_{CN} region on which the non-conservative Clostridial toxin H_{CN} region variant is based, and can be substituted for the reference Clostridial toxin H_{CN} region in any aspect of the present invention. A non-conservative Clostridial toxin H_{CN} region variant can delete one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids from the reference Clostridial toxin H_{CN} region on which the non-conservative Clostridial toxin H_{CN} region variant is based. A non-conservative Clostridial toxin H_{CN} region variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids to the reference Clostridial toxin H_{CN} region on which the non-conservative Clostridial toxin H_{CN} region variant is based. A non-conservative Clostridial toxin H_{CN} region variant may substitute one or more amino acids, two or more amino acids, three or more amino acids,

four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids or 100 or more amino acids from the reference Clostridial toxin H_{CN} region on which the non-conservative Clostridial toxin H_{CN} region variant is based. A non-conservative Clostridial toxin H_{CN} region variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin H_{CN} region on which the non-conservative Clostridial toxin H_{CN} region variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin H_{CN} region on which the non-conservative Clostridial toxin H_{CN} region variant is based. Non-limiting examples of a non-conservative Clostridial toxin H_{CN} region variant include, *e.g.*, non-conservative BoNT/A H_{CN} region variants, non-conservative BoNT/B H_{CN} region variants, non-conservative BoNT/C1 H_{CN} region variants, non-conservative BoNT/D H_{CN} region variants, non-conservative BoNT/E H_{CN} region variants, non-conservative BoNT/F H_{CN} region variants, non-conservative BoNT/G H_{CN} region variants, and non-conservative TeNT H_{CN} region variants.

[0129] As used herein, the term "Clostridial toxin H_{CN} region chimeric" means a polypeptide comprising at least a portion of a Clostridial toxin H_{CN} region and at least a portion of at least one other polypeptide to form a toxin H_{CN} region with at least one property different from the reference Clostridial toxin H_{CN} regions of Table 1, with the proviso that this Clostridial toxin H_{CN} region chimeric is still capable of further facilitating the translocation step of the intoxication process where the LC is released from intracellular vesicles into the cytoplasm of the target cell and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate.

[0130] As used herein, the term "active Clostridial toxin H_{CN} region fragment" means any of a variety of Clostridial toxin fragments comprising the H_{CN} region can be useful in aspects of the present invention with the proviso that these active fragments can further facilitate the translocation step of the intoxication process where the LC is released from intracellular vesicles into the cytoplasm of the target cell and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The H_{CN} domains from the heavy chains of Clostridial toxins are approximately 230-250 amino acids in length and comprise a translocation domain (Table 1). Additionally, while a specific amino acid positions have been identified to delineate the boundaries of the Clostridial toxin H_{CN} region (Table 1), it is well known in the art that the functional boundaries are not definitive. For example, amino-terminus of the H_{CN} domain for all naturally-occurring Clostridial toxins is the H_N domain (translocation domain). In examining the structure for BoNT/A, a random coil linker region forms a boundary between the H_N and H_{CN} domains (see FIG. 7A). The BoNT/A H_N domain appears to end with an α-helix comprising amino acids N859 to I873. Following the α-helix there is a random coil (I873 to I878) that leads into the H_{CN} domain where a β-sheet begins at position I878. The above residues define boundaries at the beginning or end of defined secondary structures and do not imply that there are not significant interactions (*i.e.*, hydrophobic, H-bond, etc.) between residues in the random coil region and one or both of the domains

that it links together. Thus, minimally an amino acid that defines the amino-terminal boundary of the BoNT/A H_{CN} domain comprises can be any amino acid present in the amino acid region Y869 to L879. Similar analysis indicates that minimally, the amino acid that defines the amino-terminal boundary of the BoNT/B H_{CN} domain can be any amino acid present in the amino acid region Y856 to L866; the amino acid that defines the amino-terminal boundary of the BoNT/C1 H_{CN} domain can be any amino acid present in the amino acid region Y864 to L874; the amino acid that defines the amino-terminal boundary of the BoNT/D H_{CN} domain can be any amino acid present in the amino acid region Y860 to L870; the amino acid that defines the amino-terminal boundary of the BoNT/E H_{CN} domain can be any amino acid present in the amino acid region F843 to L853; the amino acid that defines the amino-terminal boundary of the BoNT/F H_{CN} domain can be any amino acid present in the amino acid region L862 to L872; the amino acid that defines the amino-terminal boundary of the BoNT/G H_{CN} domain can be any amino acid present in the amino acid region Y861 to L871; and the amino acid that defines the amino-terminal boundary of the TeNT H_{CN} domain can be any amino acid present in the amino acid region I877 to L887.

[0131] Similarly, the carboxyl-terminal portion of the H_{CN} domain (*i.e.*, the fusion point between the H_{CN} and H_{CC} domains) all naturally-occurring Clostridial toxins comprises a range of amino acids. In defining the boundary of the BoNT/A H_{CN} domain as the beginning or the end of ordered secondary structure, the H_{CN} domain could end at Q1091 of an α-helix and the H_{CC} domain could begin at K1109 of a β-strand (see FIG. 7B). The intervening amino acid sequence between these two domains comprises a longer random coil but, this does not imply that the random coil is not structurally important. In fact, this random coil has a great deal of interaction with both the H_{CN} and H_{CC} domains (*i.e.*, hydrophobic and H-bonding). Thus, minimally an amino acid that defines the carboxyl-terminal boundary of the BoNT/A H_{CN} domain comprises can be any amino acid present in the amino acid region D1089 to Y1111. Similar analysis indicates that minimally, the amino acid that defines the carboxyl-terminal boundary of the BoNT/B H_{CN} domain can be any amino acid present in the amino acid region K1076 to Y1098; the amino acid that defines the carboxyl-terminal boundary of the BoNT/C1 H_{CN} domain can be any amino acid present in the amino acid region N1090 to Y1112; the amino acid that defines the carboxyl-terminal boundary of the BoNT/D H_{CN} domain can be any amino acid present in the amino acid region E1077 to Y1099; the amino acid that defines the carboxyl-terminal boundary of the BoNT/E H_{CN} domain can be any amino acid present in the amino acid region S1064 to Y1086; the amino acid that defines the carboxyl-terminal boundary of the BoNT/F H_{CN} domain can be any amino acid present in the amino acid region S1084 to Y1106; the amino acid that defines the carboxyl-terminal boundary of the BoNT/G H_{CN} domain can be any amino acid present in the amino acid region W1084 to Y1106; and the amino acid that defines the carboxyl-terminal boundary of the TeNT H_{CN} domain can be any amino acid present in the amino acid region T1106 to Y1128.

[0132] Thus, aspects of this embodiment can include Clostridial toxin H_{CN} regions comprising a translocation facilitating domain having a length of, *e.g.*, at least 200 amino acids, at least 225 amino acids, at least 250 amino acids and at least 275 amino acids. Other aspects of this embodiment can

include Clostridial toxin H_{CN} regions comprising translocation facilitating domain having a length of, *e.g.*, at most 200 amino acids, at most 225 amino acids, at most 250 amino acids and at most 275 amino acids.

[0133] Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring Clostridial toxin H_{CN} region variants and non-naturally-occurring Clostridial toxin H_{CN} region variants, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0134] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification comprises a Clostridial toxin translocation facilitating domain. In an aspect of this embodiment, a Clostridial toxin translocation facilitating domain comprises a naturally occurring Clostridial toxin H_{CN} region variant, such as, *e.g.*, a Clostridial toxin H_{CN} region isoform or a Clostridial toxin H_{CN} region subtype. In another aspect of this embodiment, a Clostridial toxin translocation domain comprises a non-naturally occurring Clostridial toxin H_{CN} region variant, such as, *e.g.*, a conservative Clostridial toxin H_{CN} region variant, a non-conservative Clostridial toxin H_{CN} region variant, a Clostridial toxin chimeric H_{CN} region, an active Clostridial toxin H_{CN} region fragment, or any combination thereof.

[0135] In another embodiment, a Clostridial toxin translocation facilitating domain comprises a BoNT/A H_{CN} region. In an aspect of this embodiment, a BoNT/A H_{CN} region comprises amino acids 874-1110 of SEQ ID NO: 1. In another aspect of this embodiment, a BoNT/A H_{CN} region comprises a naturally occurring BoNT/A H_{CN} region variant, such as, *e.g.*, a H_{CN} region from a BoNT/A isoform or a H_{CN} region from a BoNT/A subtype. In another aspect of this embodiment, a BoNT/A H_{CN} region comprises amino acids 874-1110 of a naturally occurring BoNT/A H_{CN} region variant of SEQ ID NO: 1, such as, *e.g.*, amino acids 874-1110 of a BoNT/A isoform of SEQ ID NO: 1 or amino acids 874-1110 of a BoNT/A subtype of SEQ ID NO: 1. In still another aspect of this embodiment, a BoNT/A H_{CN} region comprises a non-naturally occurring BoNT/A H_{CN} region variant, such as, *e.g.*, a conservative BoNT/A H_{CN} region variant, a non-conservative BoNT/A H_{CN} region variant, a BoNT/A chimeric H_{CN} region, an active BoNT/A H_{CN} region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A H_{CN} region comprises amino acids 874-1110 of a non-naturally occurring BoNT/A H_{CN} region variant of SEQ ID NO: 1, such as, *e.g.*, amino acids 874-1110 of a conservative BoNT/A H_{CN} region variant of SEQ ID NO: 1, amino acids 874-1110 of a non-conservative BoNT/A H_{CN} region variant of SEQ ID NO: 1, amino acids 874-1110 of an active BoNT/A H_{CN} region fragment of SEQ ID NO: 1, or any combination thereof.

[0136] In other aspects of this embodiment, a BoNT/A H_{CN} region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 874-1110 of SEQ ID NO: 1, at least 75% amino acid identity with amino acids 874-1110 of SEQ ID NO: 1, at least 80% amino acid identity with amino acids 874-1110 of SEQ ID NO: 1, at least 85% amino acid identity with amino acids 874-1110 of SEQ ID NO: 1,

at least 90% amino acid identity with amino acids 874-1110 of SEQ ID NO: 1 or at least 95% amino acid identity with amino acids 874-1110 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A H_{CN} region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 874-1110 of SEQ ID NO: 1, at most 75% amino acid identity with amino acids 874-1110 of SEQ ID NO: 1, at most 80% amino acid identity with amino acids 874-1110 of SEQ ID NO: 1, at most 85% amino acid identity with amino acids 874-1110 of SEQ ID NO: 1, at most 90% amino acid identity with amino acids 874-1110 of SEQ ID NO: 1 or at most 95% amino acid identity with amino acids 874-1110 of SEQ ID NO: 1.

[0137] In other aspects of this embodiment, a BoNT/A H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 874-1110 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid substitutions relative to amino acids 874-1110 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 874-1110 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 874-1110 of SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 874-1110 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 874-1110 of SEQ ID NO: 1.

[0138] In other aspects of this embodiment, a BoNT/A H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 874-1110 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 874-1110 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 874-1110 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 874-1110 of SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 874-1110 of SEQ ID NO: 1. In

other aspects of this embodiment, a BoNT/A H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 874-1110 of SEQ ID NO: 1.

[0139] In another embodiment, a Clostridial toxin translocation facilitating domain comprises a BoNT/B H_{CN} region. In an aspect of this embodiment, a BoNT/B H_{CN} region comprises amino acids 861-1097of SEQ ID NO: 2. In another aspect of this embodiment, a BoNT/B H_{CN} region comprises a naturally occurring BoNT/B H_{CN} region variant, such as, *e.g.*, a H_{CN} region from a BoNT/B isoform or a H_{CN} region from a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B H_{CN} region comprises amino acids 861-1097of a naturally occurring BoNT/B H_{CN} region variant of SEQ ID NO: 2, such as, *e.g.*, amino acids 861-1097of a BoNT/B isoform of SEQ ID NO: 2 or amino acids 861-1097of a BoNT/B subtype of SEQ ID NO: 2. In still another aspect of this embodiment, a BoNT/B H_{CN} region comprises a non-naturally occurring BoNT/B H_{CN} region variant, such as, *e.g.*, a conservative BoNT/B H_{CN} region variant, a non-conservative BoNT/B H_{CN} region variant, a BoNT/B chimeric H_{CN} region, an active BoNT/B H_{CN} region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B H_{CN} region comprises amino acids 861-1097of a non-naturally occurring BoNT/B H_{CN} region variant of SEQ ID NO: 2, such as, *e.g.*, amino acids 861-1097of a conservative BoNT/B H_{CN} region variant of SEQ ID NO: 2, amino acids 861-1097of a non-conservative BoNT/B H_{CN} region variant of SEQ ID NO: 2, amino acids 861-1097of an active BoNT/B H_{CN} region fragment of SEQ ID NO: 2, or any combination thereof.

[0140] In other aspects of this embodiment, a BoNT/B H_{CN} region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 861-1097of SEQ ID NO: 2, at least 75% amino acid identity with amino acids 861-1097of SEQ ID NO: 2, at least 80% amino acid identity with amino acids 861-1097of SEQ ID NO: 2, at least 85% amino acid identity with amino acids 861-1097of SEQ ID NO: 2, at least 90% amino acid identity with amino acids 861-1097of SEQ ID NO: 2 or at least 95% amino acid identity with amino acids 861-1097of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B H_{CN} region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 861-1097of SEQ ID NO: 2, at most 75% amino acid identity with amino acids 861-1097of SEQ ID NO: 2, at most 80% amino acid identity with amino acids 861-1097of SEQ ID NO: 2, at most 85% amino acid identity with amino acids 861-1097of SEQ ID NO: 2, at most 90% amino acid identity with amino acids 861-1097of SEQ ID NO: 2 or at most 95% amino acid identity with amino acids 861-1097of SEQ ID NO: 2.

[0141] In other aspects of this embodiment, a BoNT/B H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid substitutions relative to amino acids 861-1097of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid substitutions relative to amino acids 861-1097of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B H_{CN} region comprises a

polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 861-1097of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 861-1097of SEQ ID NO: 2. In still other aspects of this embodiment, a BoNT/B H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 861-1097of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 861-1097of SEQ ID NO: 2.

[0142] In other aspects of this embodiment, a BoNT/B H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 861-1097of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 861-1097of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 861-1097of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 861-1097of SEQ ID NO: 2. In still other aspects of this embodiment, a BoNT/B H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 861-1097of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 861-1097of SEQ ID NO: 2.

[0143] In another embodiment, a Clostridial toxin translocation facilitating domain comprises a BoNT/C1 H_{CN} region. In an aspect of this embodiment, a BoNT/C1 H_{CN} region comprises amino acids 869-1111 of SEQ ID NO: 3. In another aspect of this embodiment, a BoNT/C1 H_{CN} region comprises a naturally occurring BoNT/C1 H_{CN} region variant, such as, *e.g.*, a H_{CN} region from a BoNT/C1 isoform or a H_{CN} region from a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 H_{CN} region comprises amino acids 869-1111 of a naturally occurring BoNT/C1 H_{CN} region variant of SEQ ID NO: 3, such as, *e.g.*, amino acids 869-1111 of a BoNT/C1 isoform of SEQ ID NO: 3 or amino acids 869-1111 of a BoNT/C1 subtype of SEQ ID NO: 3. In still another aspect of this embodiment, a BoNT/C1 H_{CN} region comprises a non-naturally occurring BoNT/C1 H_{CN} region variant, such as, *e.g.*, a conservative BoNT/C1 H_{CN} region variant, a non-conservative BoNT/C1 H_{CN} region variant, a BoNT/C1 chimeric H_{CN} region, an active BoNT/C1 H_{CN} region fragment, or any combination thereof. In still another aspect of this

embodiment, a BoNT/C1 H_{CN} region comprises amino acids 869-1111 of a non-naturally occurring BoNT/C1 H_{CN} region variant of SEQ ID NO: 3, such as, *e.g.*, amino acids 869-1111 of a conservative BoNT/C1 H_{CN} region variant of SEQ ID NO: 3, amino acids 869-1111 of a non-conservative BoNT/C1 H_{CN} region variant of SEQ ID NO: 3, amino acids 869-1111 of an active BoNT/C1 H_{CN} region fragment of SEQ ID NO: 3, or any combination thereof.

[0144] In other aspects of this embodiment, a BoNT/C1 H_{CN} region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 869-1111 of SEQ ID NO: 3, at least 75% amino acid identity with amino acids 869-1111 of SEQ ID NO: 3, at least 80% amino acid identity with amino acids 869-1111 of SEQ ID NO: 3, at least 85% amino acid identity with amino acids 869-1111 of SEQ ID NO: 3, at least 90% amino acid identity with amino acids 869-1111 of SEQ ID NO: 3 or at least 95% amino acid identity with amino acids 869-1111 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 H_{CN} region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 869-1111 of SEQ ID NO: 3, at most 75% amino acid identity with amino acids 869-1111 of SEQ ID NO: 3, at most 80% amino acid identity with amino acids 869-1111 of SEQ ID NO: 3, at most 85% amino acid identity with amino acids 869-1111 of SEQ ID NO: 3, at most 90% amino acid identity with amino acids 869-1111 of SEQ ID NO: 3 or at most 95% amino acid identity with amino acids 869-1111 of SEQ ID NO: 3.

[0145] In other aspects of this embodiment, a BoNT/C1 H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 869-1111 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid substitutions relative to amino acids 869-1111 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 869-1111 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 869-1111 of SEQ ID NO: 3. In still other aspects of this embodiment, a BoNT/C1 H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 869-1111 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 869-1111 of SEQ ID NO: 3.

[0146] In other aspects of this embodiment, a BoNT/C1 H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 869-1111 of SEQ ID NO: 3. In other aspects of this embodiment, a

BoNT/C1 H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 869-1111 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 869-1111 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 869-1111 of SEQ ID NO: 3. In still other aspects of this embodiment, a BoNT/C1 H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 869-1111 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 869-1111 of SEQ ID NO: 3.

[0147] In another embodiment, a Clostridial toxin translocation facilitating domain comprises a BoNT/D H_{CN} region. In an aspect of this embodiment, a BoNT/D H_{CN} region comprises amino acids 865-1098 of SEQ ID NO: 4. In another aspect of this embodiment, a BoNT/D H_{CN} region comprises a naturally occurring BoNT/D H_{CN} region variant, such as, *e.g.*, a H_{CN} region from a BoNT/D isoform or a H_{CN} region from a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D H_{CN} region comprises amino acids 865-1098 of a naturally occurring BoNT/D H_{CN} region variant of SEQ ID NO: 4, such as, *e.g.*, amino acids 865-1098 of a BoNT/D isoform of SEQ ID NO: 4 or amino acids 865-1098 of a BoNT/D subtype of SEQ ID NO: 4. In still another aspect of this embodiment, a BoNT/D H_{CN} region comprises a non-naturally occurring BoNT/D H_{CN} region variant, such as, *e.g.*, a conservative BoNT/D H_{CN} region variant, a non-conservative BoNT/D H_{CN} region variant, a BoNT/D chimeric H_{CN} region, an active BoNT/D H_{CN} region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D H_{CN} region comprises amino acids 865-1098 of a non-naturally occurring BoNT/D H_{CN} region variant of SEQ ID NO: 4, such as, *e.g.*, amino acids 865-1098 of a conservative BoNT/D H_{CN} region variant of SEQ ID NO: 4, amino acids 865-1098 of a non-conservative BoNT/D H_{CN} region variant of SEQ ID NO: 4, amino acids 865-1098 of an active BoNT/D H_{CN} region fragment of SEQ ID NO: 4, or any combination thereof.

[0148] In other aspects of this embodiment, a BoNT/D H_{CN} region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 865-1098 of SEQ ID NO: 4, at least 75% amino acid identity with amino acids 865-1098 of SEQ ID NO: 4, at least 80% amino acid identity with amino acids 865-1098 of SEQ ID NO: 4, at least 85% amino acid identity with amino acids 865-1098 of SEQ ID NO: 4, at least 90% amino acid identity with amino acids 865-1098 of SEQ ID NO: 4 or at least 95% amino acid identity with amino acids 865-1098 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D H_{CN} region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 865-1098 of SEQ ID NO: 4, at most 75% amino acid identity with amino acids 865-1098 of SEQ ID NO: 4, at most 80% amino acid identity with amino acids 865-1098 of SEQ ID NO: 4, at most 85% amino acid

identity with amino acids 865-1098 of SEQ ID NO: 4, at most 90% amino acid identity with amino acids 865-1098 of SEQ ID NO: 4 or at most 95% amino acid identity with amino acids 865-1098 of SEQ ID NO: 4.

[0149] In other aspects of this embodiment, a BoNT/D H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 865-1098 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid substitutions relative to amino acids 865-1098 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 865-1098 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 865-1098 of SEQ ID NO: 4. In still other aspects of this embodiment, a BoNT/D H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 865-1098 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 865-1098 of SEQ ID NO: 4.

[0150] In other aspects of this embodiment, a BoNT/D H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 865-1098 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 865-1098 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 865-1098 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 865-1098 of SEQ ID NO: 4. In still other aspects of this embodiment, a BoNT/D H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 865-1098 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 865-1098 of SEQ ID NO: 4.

[0151] In another embodiment, a Clostridial toxin translocation facilitating domain comprises a BoNT/E H_{CN} region. In an aspect of this embodiment, a BoNT/E H_{CN} region comprises amino acids 848-1085 of SEQ ID NO: 5. In another aspect of this embodiment, a BoNT/E H_{CN} region comprises a naturally occurring BoNT/E H_{CN} region variant, such as, *e.g.*, a H_{CN} region from a BoNT/E isoform or a H_{CN} region from a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E H_{CN} region comprises amino acids 848-1085 of a naturally occurring BoNT/E H_{CN} region variant of SEQ ID NO: 5, such as, *e.g.*, amino acids 848-1085 of a BoNT/E isoform of SEQ ID NO: 5 or amino acids 848-1085 of a BoNT/E subtype of SEQ ID NO: 5. In still another aspect of this embodiment, a BoNT/E H_{CN} region comprises a non-naturally occurring BoNT/E H_{CN} region variant, such as, *e.g.*, a conservative BoNT/E H_{CN} region variant, a non-conservative BoNT/E H_{CN} region variant, a BoNT/E chimeric H_{CN} region, an active BoNT/E H_{CN} region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E H_{CN} region comprises amino acids 848-1085 of a non-naturally occurring BoNT/E H_{CN} region variant of SEQ ID NO: 5, such as, *e.g.*, amino acids 848-1085 of a conservative BoNT/E H_{CN} region variant of SEQ ID NO: 5, amino acids 848-1085 of a non-conservative BoNT/E H_{CN} region variant of SEQ ID NO: 5, amino acids 848-1085 of an active BoNT/E H_{CN} region fragment of SEQ ID NO: 5, or any combination thereof.

[0152] In other aspects of this embodiment, a BoNT/E H_{CN} region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 848-1085 of SEQ ID NO: 5, at least 75% amino acid identity with amino acids 848-1085 of SEQ ID NO: 5, at least 80% amino acid identity with amino acids 848-1085 of SEQ ID NO: 5, at least 85% amino acid identity with amino acids 848-1085 of SEQ ID NO: 5, at least 90% amino acid identity with amino acids 848-1085 of SEQ ID NO: 5 or at least 95% amino acid identity with amino acids 848-1085 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E H_{CN} region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 848-1085 of SEQ ID NO: 5, at most 75% amino acid identity with amino acids 848-1085 of SEQ ID NO: 5, at most 80% amino acid identity with amino acids 848-1085 of SEQ ID NO: 5, at most 85% amino acid identity with amino acids 848-1085 of SEQ ID NO: 5, at most 90% amino acid identity with amino acids 848-1085 of SEQ ID NO: 5 or at most 95% amino acid identity with amino acids 848-1085 of SEQ ID NO: 5.

[0153] In other aspects of this embodiment, a BoNT/E H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 848-1085 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid substitutions relative to amino acids 848-1085 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 848-1085 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino

acid deletions relative to amino acids 848-1085 of SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/E H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 848-1085 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 848-1085 of SEQ ID NO: 5.

[0154] In other aspects of this embodiment, a BoNT/E H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 848-1085 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 848-1085 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 848-1085 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 848-1085 of SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/E H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 848-1085 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 848-1085 of SEQ ID NO: 5.

[0155] In another embodiment, a Clostridial toxin translocation facilitating domain comprises a BoNT/F H_{CN} region. In an aspect of this embodiment, a BoNT/F H_{CN} region comprises amino acids 867-1105 of SEQ ID NO: 6. In another aspect of this embodiment, a BoNT/F H_{CN} region comprises a naturally occurring BoNT/F H_{CN} region variant, such as, *e.g.*, a H_{CN} region from a BoNT/F isoform or a H_{CN} region from a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F H_{CN} region comprises amino acids 867-1105 of a naturally occurring BoNT/F H_{CN} region variant of SEQ ID NO: 6, such as, *e.g.*, amino acids 867-1105 of a BoNT/F isoform of SEQ ID NO: 6 or amino acids 867-1105 of a BoNT/F subtype of SEQ ID NO: 6. In still another aspect of this embodiment, a BoNT/F H_{CN} region comprises a non-naturally occurring BoNT/F H_{CN} region variant, such as, *e.g.*, a conservative BoNT/F H_{CN} region variant, a non-conservative BoNT/F H_{CN} region variant, a BoNT/F chimeric H_{CN} region, an active BoNT/F H_{CN} region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F H_{CN} region comprises amino acids 867-1105 of a non-naturally occurring BoNT/F H_{CN} region variant of SEQ ID NO: 6, such as, *e.g.*, amino acids 867-1105 of a conservative BoNT/F H_{CN} region variant of SEQ ID NO: 6, amino acids 867-1105 of a non-conservative BoNT/F H_{CN} region variant of SEQ ID NO: 6, amino acids 867-1105 of an active BoNT/F H_{CN} region fragment of SEQ ID NO: 6, or any combination thereof.

[0156] In other aspects of this embodiment, a BoNT/F H_{CN} region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 867-1105 of SEQ ID NO: 6, at least 75% amino acid identity with amino acids 867-1105 of SEQ ID NO: 6, at least 80% amino acid identity with amino acids 867-1105 of SEQ ID NO: 6, at least 85% amino acid identity with amino acids 867-1105 of SEQ ID NO: 6, at least 90% amino acid identity with amino acids 867-1105 of SEQ ID NO: 6 or at least 95% amino acid identity with amino acids 867-1105 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F H_{CN} region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 867-1105 of SEQ ID NO: 6, at most 75% amino acid identity with amino acids 867-1105 of SEQ ID NO: 6, at most 80% amino acid identity with amino acids 867-1105 of SEQ ID NO: 6, at most 85% amino acid identity with amino acids 867-1105 of SEQ ID NO: 6, at most 90% amino acid identity with amino acids 867-1105 of SEQ ID NO: 6 or at most 95% amino acid identity with amino acids 867-1105 of SEQ ID NO: 6.

[0157] In other aspects of this embodiment, a BoNT/F H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 867-1105 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid substitutions relative to amino acids 867-1105 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 867-1105 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 867-1105 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/F H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 867-1105 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 867-1105 of SEQ ID NO: 6.

[0158] In other aspects of this embodiment, a BoNT/F H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 867-1105 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 867-1105 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 867-1105 of SEQ ID NO: 6. In other aspects

of this embodiment, a BoNT/F H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 867-1105 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/F H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 867-1105 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 867-1105 of SEQ ID NO: 6.

[0159] In another embodiment, a Clostridial toxin translocation facilitating domain comprises a BoNT/G H_{CN} region. In an aspect of this embodiment, a BoNT/G H_{CN} region comprises amino acids 866-1105 of SEQ ID NO: 7. In another aspect of this embodiment, a BoNT/G H_{CN} region comprises a naturally occurring BoNT/G H_{CN} region variant, such as, *e.g.*, a H_{CN} region from a BoNT/G isoform or a H_{CN} region from a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G H_{CN} region comprises amino acids 866-1105 of a naturally occurring BoNT/G H_{CN} region variant of SEQ ID NO: 7, such as, *e.g.*, amino acids 866-1105 of a BoNT/G isoform of SEQ ID NO: 7 or amino acids 866-1105 of a BoNT/G subtype of SEQ ID NO: 7. In still another aspect of this embodiment, a BoNT/G H_{CN} region comprises a non-naturally occurring BoNT/G H_{CN} region variant, such as, *e.g.*, a conservative BoNT/G H_{CN} region variant, a non-conservative BoNT/G H_{CN} region variant, a BoNT/G chimeric H_{CN} region, an active BoNT/G H_{CN} region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G H_{CN} region comprises amino acids 866-1105 of a non-naturally occurring BoNT/G H_{CN} region variant of SEQ ID NO: 7, such as, *e.g.*, amino acids 866-1105 of a conservative BoNT/G H_{CN} region variant of SEQ ID NO: 7, amino acids 866-1105 of a non-conservative BoNT/G H_{CN} region variant of SEQ ID NO: 7, amino acids 866-1105 of an active BoNT/G H_{CN} region fragment of SEQ ID NO: 7, or any combination thereof.

[0160] In other aspects of this embodiment, a BoNT/G H_{CN} region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 866-1105 of SEQ ID NO: 7, at least 75% amino acid identity with amino acids 866-1105 of SEQ ID NO: 7, at least 80% amino acid identity with amino acids 866-1105 of SEQ ID NO: 7, at least 85% amino acid identity with amino acids 866-1105 of SEQ ID NO: 7, at least 90% amino acid identity with amino acids 866-1105 of SEQ ID NO: 7 or at least 95% amino acid identity with amino acids 866-1105 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G H_{CN} region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 866-1105 of SEQ ID NO: 7, at most 75% amino acid identity with amino acids 866-1105 of SEQ ID NO: 7, at most 80% amino acid identity with amino acids 866-1105 of SEQ ID NO: 7, at most 85% amino acid identity with amino acids 866-1105 of SEQ ID NO: 7, at most 90% amino acid identity with amino acids 866-1105 of SEQ ID NO: 7 or at most 95% amino acid identity with amino acids 866-1105 of SEQ ID NO: 7.

[0161] In other aspects of this embodiment, a BoNT/G H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 866-1105 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid substitutions relative to amino acids 866-1105 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 866-1105 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 866-1105 of SEQ ID NO: 7. In still other aspects of this embodiment, a BoNT/G H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 866-1105 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 866-1105 of SEQ ID NO: 7.

[0162] In other aspects of this embodiment, a BoNT/G H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 866-1105 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 866-1105 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 866-1105 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 866-1105 of SEQ ID NO: 7. In still other aspects of this embodiment, a BoNT/G H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 866-1105 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 866-1105 of SEQ ID NO: 7.

[0163] In another embodiment, a Clostridial toxin translocation facilitating domain comprises a TeNT H_{CN} region. In an aspect of this embodiment, a TeNT H_{CN} region comprises amino acids 882-1127 of SEQ ID NO: 8. In another aspect of this embodiment, a TeNT H_{CN} region comprises a naturally occurring TeNT H_{CN} region variant, such as, *e.g.*, a H_{CN} region from a TeNT isoform or a H_{CN} region from a TeNT subtype. In another aspect of this embodiment, a TeNT H_{CN} region comprises amino acids 882-1127 of a naturally

occurring TeNT H_{CN} region variant of SEQ ID NO: 8, such as, *e.g.*, amino acids 882-1127 of a TeNT isoform of SEQ ID NO: 8 or amino acids 882-1127 of a TeNT subtype of SEQ ID NO: 8. In still another aspect of this embodiment, a TeNT H_{CN} region comprises a non-naturally occurring TeNT H_{CN} region variant, such as, *e.g.*, a conservative TeNT H_{CN} region variant, a non-conservative TeNT H_{CN} region variant, a TeNT chimeric H_{CN} region, an active TeNT H_{CN} region fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT H_{CN} region comprises amino acids 882-1127 of a non-naturally occurring TeNT H_{CN} region variant of SEQ ID NO: 8, such as, *e.g.*, amino acids 882-1127 of a conservative TeNT H_{CN} region variant of SEQ ID NO: 8, amino acids 882-1127 of a non-conservative TeNT H_{CN} region variant of SEQ ID NO: 8, amino acids 882-1127 of an active TeNT H_{CN} region fragment of SEQ ID NO: 8, or any combination thereof.

[0164] In other aspects of this embodiment, a TeNT H_{CN} region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 882-1127 of SEQ ID NO: 8, at least 75% amino acid identity with amino acids 882-1127 of SEQ ID NO: 8, at least 80% amino acid identity with amino acids 882-1127 of SEQ ID NO: 8, at least 85% amino acid identity with amino acids 882-1127 of SEQ ID NO: 8, at least 90% amino acid identity with amino acids 882-1127 of SEQ ID NO: 8 or at least 95% amino acid identity with amino acids 882-1127 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT H_{CN} region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 882-1127 of SEQ ID NO: 8, at most 75% amino acid identity with amino acids 882-1127 of SEQ ID NO: 8, at most 80% amino acid identity with amino acids 882-1127 of SEQ ID NO: 8, at most 85% amino acid identity with amino acids 882-1127 of SEQ ID NO: 8, at most 90% amino acid identity with amino acids 882-1127 of SEQ ID NO: 8 or at most 95% amino acid identity with amino acids 882-1127 of SEQ ID NO: 8.

[0165] In other aspects of this embodiment, a TeNT H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 882-1127 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid substitutions relative to amino acids 882-1127 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 882-1127 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid deletions relative to amino acids 882-1127 of SEQ ID NO: 8. In still other aspects of this embodiment, a TeNT H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 882-1127 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_{CN} region comprises a polypeptide

having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 non-contiguous amino acid additions relative to amino acids 882-1127 of SEQ ID NO: 8.

[0166] In other aspects of this embodiment, a TeNT H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 882-1127 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid substitutions relative to amino acids 882-1127 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 882-1127 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid deletions relative to amino acids 882-1127 of SEQ ID NO: 8. In still other aspects of this embodiment, a TeNT H_{CN} region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 882-1127 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_{CN} region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50 or 100 contiguous amino acid additions relative to amino acids 882-1127 of SEQ ID NO: 8.

[0167] The fusion of the membrane of enveloped viruses to a cellular membrane is an essential step in the release of the viral capsule into the cytoplasm of the host cell. This fusion event is mediated by a fusogenic peptide segment present in viral glycoproteins located on the viral membrane and involves either a pH-dependent or pH-independent process, see, *e.g.*, Frederick M. Hughson, *Structural Characterization of Viral Fusion Proteins*, 5(3) Curr. Biol. 265-274 (1995); Trudy G. Morrison, *Structure and Function of a Paramyxovirus Fusion Protein*, 1614(1) Biochim. Biophys. Acta. 73-84; David J. Schibli and Winfried Weissenhorn, *Class I and Class II Viral Fusion Protein Structures Reveal Similar Principles in Membrane Fusion*, 21(6) Mol. Membr. Biol. 361-371 (2004). The fusogenic peptide domain comprises a hydrophobic, glycine-rich peptide of approximately 20-30 amino acids that assist in the insertion of the viral capsule into a cellular membrane. Thus, an enveloped virus fusogenic peptide domain can be useful as a translocation facilitating domain.

[0168] An enveloped virus fusogenic peptide domain includes, without limitation, naturally occurring enveloped virus fusogenic peptide domain variants, such as, *e.g.*, enveloped virus fusogenic peptide domain isoforms and enveloped virus fusogenic peptide domain subtypes; non-naturally occurring enveloped virus fusogenic peptide domain variants, such as, *e.g.*, conservative enveloped virus fusogenic peptide domain variants, non-conservative enveloped virus fusogenic peptide domain variants, enveloped virus fusogenic peptide domain chimerics, active enveloped virus fusogenic peptide domain fragments thereof, or any combination thereof.

[0169] As used herein, the term "enveloped virus fusogenic peptide domain variant," whether naturally-occurring or non-naturally-occurring, means an enveloped virus fusogenic peptide domain that has at least one amino acid change from the corresponding region of the disclosed reference sequences and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, all Clostridial toxin H_{CN} region variants disclosed in the present specification are capable of further facilitating the translocation step of the intoxication process that mediates Clostridial toxin light chain translocation. As non-limiting examples, an Influenza virus A fusogenic peptide domain variant comprising SEQ ID NO: 194 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 194; a Semliki Forest virus fusogenic peptide domain variant comprising SEQ ID NO: 199 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 199; an Eastern equine encephalitis virus fusogenic peptide domain variant comprising SEQ ID NO: 201 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 201; a Venezuelan equine encephalitis virus fusogenic peptide domain variant comprising SEQ ID NO: 209 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 209; a Vesicular stomatitis virus fusogenic peptide domain variant comprising SEQ ID NO: 226 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 226; a Sendai virus fusogenic peptide domain variant comprising SEQ ID NO: 237 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 237; a Canine distemper virus fusogenic peptide domain variant comprising SEQ ID NO: 244 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 244; a Newcastle disease virus fusogenic peptide domain variant comprising SEQ ID NO: 254 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 254; and a Hendra virus fusogenic peptide domain variant comprising SEQ ID NO: 267 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 267.

[0170] It is recognized by those of skill in the art that for each enveloped virus there can be naturally occurring fusogenic peptide domain variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, at least five naturally-occurring variants of the fusogenic peptide domain present in the Influenza A virus haemagglutinin are known (SEQ ID NO: 194 to SEQ ID NO: 198); at least six naturally-occurring variants of the fusogenic peptide domain present in the Eastern equine encephalitis virus E1 protein are known (SEQ ID NO: 201 to SEQ ID NO: 206); at least eight naturally-occurring variants of the fusogenic peptide domain present in the Venezuelan equine encephalitis virus E1 protein are known (SEQ ID NO: 209 to SEQ ID NO: 216); at least seven naturally-occurring variants of the fusogenic peptide domain present in the Vesicular stomatitis virus (VSV) glycoprotein G are known (SEQ ID NO: 226 to SEQ ID NO: 232); and at least eleven naturally-occurring

variants of the fusogenic peptide domain present in the Newcastle disease virus F protein are known (SEQ ID NO: 254 to SEQ ID NO: 264). As used herein, the term "enveloped virus fusogenic peptide domain variant" means any enveloped virus fusogenic peptide domain produced by a naturally-occurring process, including, without limitation, enveloped virus fusogenic peptide domain isoforms produced from alternatively-spliced transcripts, enveloped virus fusogenic peptide domain isoforms produced by spontaneous mutation and enveloped virus fusogenic peptide domain subtypes. A naturally occurring enveloped virus fusogenic peptide domain variant can function in substantially the same manner as the reference enveloped virus fusogenic peptide domain on which the naturally occurring enveloped virus fusogenic peptide domain variant is based, and can be substituted for the reference enveloped virus fusogenic peptide domain in any aspect of the present invention. A naturally occurring enveloped virus fusogenic peptide domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids or ten or more amino acids from the reference enveloped virus fusogenic peptide domain on which the naturally occurring enveloped virus fusogenic peptide domain is based. A naturally occurring enveloped virus fusogenic peptide domain variant can also substitute at least 2 contiguous amino acids, at least 3 contiguous amino acids, at least 4 contiguous amino acids or at least 5 contiguous amino acids from the reference enveloped virus fusogenic peptide domain on which the naturally occurring enveloped virus fusogenic peptide domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference enveloped virus fusogenic peptide domain on which the naturally occurring enveloped virus fusogenic peptide domain variant is based.

[0171] A non-limiting examples of a naturally occurring enveloped virus fusogenic peptide domain variant is an enveloped virus fusogenic peptide domain isoform such as, *e.g.*, an influenzavirus fusogenic peptide domain isoform, an alphavirus fusogenic peptide domain isoform, a vesiculovirus fusogenic peptide domain isoform, a respirovirus fusogenic peptide domain isoform, a morbillivirus fusogenic peptide domain isoform, an avulavirus fusogenic peptide domain isoform, a henipavirus fusogenic peptide domain isoform, a metapneumovirus fusogenic peptide domain isoform and a foamy virus fusogenic peptide domain isoform. An enveloped virus fusogenic peptide domain isoform can function in substantially the same manner as the reference enveloped virus fusogenic peptide domain on which the enveloped virus fusogenic peptide domain isoform is based, and can be substituted for the reference enveloped virus fusogenic peptide domain in any aspect of the present invention.

[0172] A non-limiting examples of a naturally occurring enveloped virus fusogenic peptide domain variant is an enveloped virus fusogenic peptide domain subtype such as, *e.g.*, an influenzavirus fusogenic peptide domain subtype, an alphavirus fusogenic peptide domain subtype, a vesiculovirus fusogenic peptide domain subtype, a respirovirus fusogenic peptide domain subtype, a morbillivirus fusogenic peptide domain subtype, an avulavirus fusogenic peptide domain subtype, a henipavirus fusogenic peptide domain subtype, a metapneumovirus fusogenic peptide domain subtype and a foamy virus

fusogenic peptide domain subtype. An enveloped virus fusogenic peptide domain subtype can function in substantially the same manner as the reference enveloped virus fusogenic peptide domain on which the enveloped virus fusogenic peptide domain subtype is based, and can be substituted for the reference enveloped virus fusogenic peptide domain in any aspect of the present invention.

[0173] As used herein, the term "non-naturally occurring enveloped virus fusogenic peptide domain variant" means any enveloped virus fusogenic peptide domain produced with the aid of human manipulation, including, without limitation, enveloped virus fusogenic peptide domains produced by genetic engineering using random mutagenesis or rational design and enveloped virus fusogenic peptide domains produced by chemical synthesis. Non-limiting examples of non-naturally occurring enveloped virus fusogenic peptide domain variants include, *e.g.*, conservative enveloped virus fusogenic peptide domain variants, non-conservative enveloped virus fusogenic peptide domain variants, enveloped virus fusogenic peptide domain chimeric variants and active enveloped virus fusogenic peptide domain fragments.

[0174] As used herein, the term "conservative enveloped virus fusogenic peptide domain variant" means an enveloped virus fusogenic peptide domain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference enveloped virus fusogenic peptide domain sequence. Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative enveloped virus fusogenic peptide domain variant can function in substantially the same manner as the reference enveloped virus fusogenic peptide domain on which the conservative enveloped virus fusogenic peptide domain variant is based, and can be substituted for the reference enveloped virus fusogenic peptide domain in any aspect of the present invention. A conservative enveloped virus fusogenic peptide domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids or ten or more amino acids from the reference enveloped virus fusogenic peptide domain on which the conservative enveloped virus fusogenic peptide domain variant is based. A conservative enveloped virus fusogenic peptide domain variant can also substitute at least 2 contiguous amino acids, at least 3 contiguous amino acids, at least 4 contiguous amino acids or at least 5 contiguous amino acids from the reference enveloped virus fusogenic peptide domain on which the conservative enveloped virus fusogenic peptide domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference enveloped virus fusogenic peptide domain on which the conservative enveloped virus fusogenic peptide domain variant is based. Non-limiting examples of a conservative enveloped virus fusogenic peptide domain variant include, *e.g.*, conservative influenzavirus fusogenic peptide domain variants, conservative alphavirus fusogenic peptide domain variants, conservative vesiculovirus fusogenic peptide domain variants, conservative respirovirus fusogenic peptide domain variants, conservative

morbillivirus fusogenic peptide domain variants, conservative avulavirus fusogenic peptide domain variants, conservative henipavirus fusogenic peptide domain variants, conservative metapneumovirus fusogenic peptide domain variants and conservative foamy virus fusogenic peptide domain variants.

[0175] As used herein, the term "non-conservative enveloped virus fusogenic peptide domain variant" means an enveloped virus fusogenic peptide domain in which 1) at least one amino acid is deleted from the reference enveloped virus fusogenic peptide domain on which the non-conservative enveloped virus fusogenic peptide domain variant is based; 2) at least one amino acid added to the reference enveloped virus fusogenic peptide domain on which the non-conservative enveloped virus fusogenic peptide domain is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference enveloped virus fusogenic peptide domain sequence. A non-conservative enveloped virus fusogenic peptide domain variant can function in substantially the same manner as the reference enveloped virus fusogenic peptide domain on which the non-conservative enveloped virus fusogenic peptide domain variant is based, and can be substituted for the reference enveloped virus fusogenic peptide domain in any aspect of the present invention. A non-conservative enveloped virus fusogenic peptide domain variant can delete one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids or five or more amino acids from the reference enveloped virus fusogenic peptide domain on which the non-conservative enveloped virus fusogenic peptide domain variant is based. A non-conservative enveloped virus fusogenic peptide domain variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids or five or more amino acids to the reference enveloped virus fusogenic peptide domain on which the non-conservative enveloped virus fusogenic peptide domain variant is based. A non-conservative enveloped virus fusogenic peptide domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids or ten or more amino acids from the reference enveloped virus fusogenic peptide domain on which the non-conservative enveloped virus fusogenic peptide domain variant is based. A non-conservative enveloped virus fusogenic peptide domain variant can also substitute at least 2 contiguous amino acids, at least 3 contiguous amino acids, at least 4 contiguous amino acids or at least 5 contiguous amino acids from the reference enveloped virus fusogenic peptide domain on which the non-conservative enveloped virus fusogenic peptide domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference enveloped virus fusogenic peptide domain on which the non-conservative enveloped virus fusogenic peptide domain variant is based. Non-limiting examples of a non-conservative enveloped virus fusogenic peptide domain variant include, *e.g.*, non-conservative influenzavirus fusogenic peptide domain variants, non-conservative alphavirus fusogenic peptide domain variants, non-conservative vesiculovirus fusogenic peptide domain variants, non-conservative respirovirus fusogenic peptide domain variants, non-conservative morbillivirus fusogenic peptide domain variants, non-conservative avulavirus fusogenic peptide domain variants, non-conservative henipavirus fusogenic

peptide domain variants, non-conservative metapneumovirus fusogenic peptide domain variants and non-conservative foamy virus fusogenic peptide domain variants.

[0176] As used herein, the term "enveloped virus fusogenic peptide domain chimeric" means a polypeptide comprising at least a portion of an enveloped virus fusogenic peptide domain and at least a portion of at least one other polypeptide to form an enveloped virus fusogenic peptide domain with at least one property different from the reference enveloped virus fusogenic peptide domain, with the proviso that this enveloped virus fusogenic peptide domain chimeric is still capable of further facilitating the translocation step of the intoxication process where the LC is released from intracellular vesicles into the cytoplasm of the target cell and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate.

[0177] As used herein, the term "active enveloped virus fusogenic peptide domain fragment" means any of a variety of enveloped virus fusogenic peptide domain fragments that can further facilitate the translocation step of the intoxication process where the LC is released from intracellular vesicles into the cytoplasm of the target cell and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. Enveloped virus fusogenic peptide domains are approximately 15-30 amino acids in length. Thus, aspects of this embodiment can include a translocation facilitating domain comprising an active enveloped virus fusogenic peptide domain fragment having a length of, *e.g.*, at least 10 amino acids, at least 15 amino acids, at least 20 amino acids and at least 25 amino acids. Other aspects of this embodiment can include a translocation facilitating domain comprising an active enveloped virus fusogenic peptide domain fragment having a length of, *e.g.*, at most 10 amino acids, at most 15 amino acids, at most 20 amino acids and at most 25 amino acids.

[0178] Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring enveloped virus fusogenic peptide domain variants and non-naturally-occurring enveloped virus fusogenic peptide domain variants, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0179] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification comprises a Clostridial toxin translocation facilitating domain comprising an enveloped virus fusogenic peptide domain. In an aspect of this embodiment, a Clostridial toxin translocation facilitating domain comprises a naturally occurring enveloped virus fusogenic peptide domain variant, such as, *e.g.*, an enveloped virus fusogenic peptide domain isoform or an enveloped virus fusogenic peptide domain subtype. In another aspect of this embodiment, a Clostridial toxin translocation domain comprises a non-naturally occurring enveloped virus fusogenic peptide domain variant, such as, *e.g.*, a conservative enveloped virus fusogenic peptide domain variant, a non-conservative enveloped virus fusogenic peptide

domain variant, an enveloped virus fusogenic peptide domain chimeric, an active enveloped virus fusogenic peptide domain fragment, or any combination thereof.

[0180] In another embodiment, a Clostridial toxin translocation facilitating domain comprises an influenzavirus fusogenic peptide domain. In another aspect of this embodiment, an influenzavirus fusogenic peptide domain comprises a naturally occurring influenzavirus fusogenic peptide domain variant, such as, *e.g.*, an influenzavirus fusogenic peptide domain isoform or an influenzavirus fusogenic peptide domain subtype. In another aspect of this embodiment, an influenzavirus fusogenic peptide domain comprises a naturally occurring influenzavirus fusogenic peptide domain variant of SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198, such as, *e.g.*, an influenzavirus fusogenic peptide domain isoform of SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198 or an influenzavirus fusogenic peptide domain subtype of SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198. In still another aspect of this embodiment, an influenzavirus fusogenic peptide domain comprises a non-naturally occurring influenzavirus fusogenic peptide domain variant, such as, *e.g.*, a conservative influenzavirus fusogenic peptide domain variant, a non-conservative influenzavirus fusogenic peptide domain variant, an influenzavirus fusogenic peptide domain chimeric, an active influenzavirus fusogenic peptide domain fragment, or any combination thereof. In still another aspect of this embodiment, an influenzavirus fusogenic peptide domain comprises amino acids a non-naturally occurring influenzavirus fusogenic peptide domain variant of SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198, such as, *e.g.*, a conservative influenzavirus fusogenic peptide domain variant of SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198, a non-conservative influenzavirus fusogenic peptide domain variant of SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198, an active influenzavirus fusogenic peptide domain fragment of SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198, or any combination thereof.

[0181] In other aspects of this embodiment, an influenzavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198, at least 75% amino acid identity with SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198, at least 80% amino acid identity with SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198, at least 85% amino acid identity with SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198, at least 90% amino acid identity with SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198 or at least 95% amino acid identity with SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198. In yet other aspects of this embodiment, an influenzavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198, at most 75% amino acid identity with SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO:

196, SEQ ID NO: 197 or SEQ ID NO: 198, at most 80% amino acid identity with SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198, at most 85% amino acid identity with SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198, at most 90% amino acid identity with SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198 or at most 95% amino acid identity with SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198.

[0182] In other aspects of this embodiment, an influenzavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198. In other aspects of this embodiment, an influenzavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198. In yet other aspects of this embodiment, an influenzavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198. In other aspects of this embodiment, an influenzavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198. In still other aspects of this embodiment, an influenzavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198. In other aspects of this embodiment, an influenzavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198.

[0183] In other aspects of this embodiment, an influenzavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198. In other aspects of this embodiment, an influenzavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198. In yet other aspects of this embodiment, an influenzavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198. In other aspects of this embodiment, an influenzavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 194, SEQ ID NO: 195,

SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198. In still other aspects of this embodiment, an influenzavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198. In other aspects of this embodiment, an influenzavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198.

[0184] In another embodiment, a Clostridial toxin translocation facilitating domain comprises an alphavirus fusogenic peptide domain. In another aspect of this embodiment, an alphavirus fusogenic peptide domain comprises a naturally occurring alphavirus fusogenic peptide domain variant, such as, *e.g.*, an alphavirus fusogenic peptide domain isoform or an alphavirus fusogenic peptide domain subtype. In another aspect of this embodiment, an alphavirus fusogenic peptide domain comprises a naturally occurring alphavirus fusogenic peptide domain variant of SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225, such as, *e.g.*, an alphavirus fusogenic peptide domain isoform of SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225 or an alphavirus fusogenic peptide domain subtype of SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225. In still another aspect of this embodiment, an alphavirus fusogenic peptide domain comprises a non-naturally occurring alphavirus fusogenic peptide domain variant, such as, *e.g.*, a conservative alphavirus fusogenic peptide domain variant, a non-conservative alphavirus fusogenic peptide domain variant, an alphavirus fusogenic peptide domain chimeric, an active alphavirus fusogenic peptide domain fragment, or any combination thereof. In still another aspect of this embodiment, an alphavirus fusogenic peptide domain comprises amino acids a non-naturally occurring alphavirus fusogenic peptide domain variant of SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222,

SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225, such as, *e.g.*, a conservative alphavirus fusogenic peptide domain variant of SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225, a non-conservative alphavirus fusogenic peptide domain variant of SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225, an active alphavirus fusogenic peptide domain fragment of SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225, or any combination thereof.

[0185] In other aspects of this embodiment, an alphavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225, at least 75% amino acid identity with SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225, at least 80% amino acid identity with SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 or SEQ ID NO: 198, at least 85% amino acid identity with SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225, at least 90% amino acid identity with SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ

ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225 or at least 95% amino acid identity with SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225. In yet other aspects of this embodiment, an alphavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225, at most 75% amino acid identity with SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225, at most 80% amino acid identity with SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225, at most 85% amino acid identity with SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225, at most 90% amino acid identity with SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225 or at most 95% amino acid identity with SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225.

[0186] In other aspects of this embodiment, an alphavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225. In other aspects of this embodiment, an alphavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225. In yet other aspects of this embodiment, an alphavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225. In other aspects of this embodiment, an alphavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225. In still other aspects of this embodiment, an alphavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225. In other aspects of this embodiment, an alphavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO:

204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225.

[0187] In other aspects of this embodiment, an alphavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225. In other aspects of this embodiment, an alphavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225. In yet other aspects of this embodiment, an alphavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225. In other aspects of this embodiment, an alphavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225. In still other aspects of this embodiment, an alphavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221,

SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225. In other aspects of this embodiment, an alphavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 or SEQ ID NO: 225.

[0188] In another embodiment, a Clostridial toxin translocation facilitating domain comprises a vesiculovirus fusogenic peptide domain. In another aspect of this embodiment, a vesiculovirus fusogenic peptide domain comprises a naturally occurring vesiculovirus fusogenic peptide domain variant, such as, *e.g.*, a vesiculovirus fusogenic peptide domain isoform or a vesiculovirus fusogenic peptide domain subtype. In another aspect of this embodiment, a vesiculovirus fusogenic peptide domain comprises a naturally occurring vesiculovirus fusogenic peptide domain variant of SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236, such as, *e.g.*, a vesiculovirus fusogenic peptide domain isoform of SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236 or a vesiculovirus fusogenic peptide domain subtype of SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236. In still another aspect of this embodiment, a vesiculovirus fusogenic peptide domain comprises a non-naturally occurring vesiculovirus fusogenic peptide domain variant, such as, *e.g.*, a conservative vesiculovirus fusogenic peptide domain variant, a non-conservative vesiculovirus fusogenic peptide domain variant, a vesiculovirus fusogenic peptide domain chimeric, an active vesiculovirus fusogenic peptide domain fragment, or any combination thereof. In still another aspect of this embodiment, a vesiculovirus fusogenic peptide domain comprises amino acids a non-naturally occurring vesiculovirus fusogenic peptide domain variant of SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236, such as, *e.g.*, a conservative vesiculovirus fusogenic peptide domain variant of SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236, a non-conservative vesiculovirus fusogenic peptide domain variant of SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236, an active vesiculovirus fusogenic peptide domain fragment of SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236, or any combination thereof.

[0189] In other aspects of this embodiment, a vesiculovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236, at least 75% amino acid identity with SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236, at least 80% amino acid identity with SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236, at least 85% amino acid identity with SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236, at least 90% amino acid identity with SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236 or at least 95% amino acid identity with SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236. In yet other aspects of this embodiment, a vesiculovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236, at most 75% amino acid identity with SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236, at most 80% amino acid identity with SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236, at most 85% amino acid identity with SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236, at most 90% amino acid identity with SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236 or at most 95% amino acid identity with SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236.

[0190] In other aspects of this embodiment, a vesiculovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236. In other aspects of this embodiment, a vesiculovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID

NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236. In yet other aspects of this embodiment, a vesiculovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236. In other aspects of this embodiment, a vesiculovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236. In still other aspects of this embodiment, a vesiculovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236. In other aspects of this embodiment, a vesiculovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236.

[0191] In other aspects of this embodiment, a vesiculovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236. In other aspects of this embodiment, a vesiculovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236. In yet other aspects of this embodiment, a vesiculovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236. In other aspects of this embodiment, a vesiculovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236. In still other aspects of this embodiment, a vesiculovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236. In other aspects of this

embodiment, a vesiculovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 or SEQ ID NO: 236.

[0192] In another embodiment, a Clostridial toxin translocation facilitating domain comprises a respirovirus fusogenic peptide domain. In another aspect of this embodiment, a respirovirus fusogenic peptide domain comprises a naturally occurring respirovirus fusogenic peptide domain variant, such as, *e.g.*, a respirovirus fusogenic peptide domain isoform or a respirovirus fusogenic peptide domain subtype. In another aspect of this embodiment, a respirovirus fusogenic peptide domain comprises a naturally occurring respirovirus fusogenic peptide domain variant of SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243, such as, *e.g.*, a respirovirus fusogenic peptide domain isoform of SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243 or a respirovirus fusogenic peptide domain subtype of SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243. In still another aspect of this embodiment, a respirovirus fusogenic peptide domain comprises a non-naturally occurring respirovirus fusogenic peptide domain variant, such as, *e.g.*, a conservative respirovirus fusogenic peptide domain variant, a non-conservative respirovirus fusogenic peptide domain variant, a respirovirus fusogenic peptide domain chimeric, an active respirovirus fusogenic peptide domain fragment, or any combination thereof. In still another aspect of this embodiment, a respirovirus fusogenic peptide domain comprises amino acids a non-naturally occurring respirovirus fusogenic peptide domain variant of SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243, such as, *e.g.*, a conservative respirovirus fusogenic peptide domain variant of SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243, a non-conservative respirovirus fusogenic peptide domain variant of SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243, an active respirovirus fusogenic peptide domain fragment of SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243, or any combination thereof.

[0193] In other aspects of this embodiment, a respirovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243, at least 75% amino acid identity with SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243, at least 80% amino acid identity with SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243, at least 85% amino acid identity with SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243, at least 90% amino acid identity with SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID

NO: 243 or at least 95% amino acid identity with SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243. In yet other aspects of this embodiment, a respirovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243, at most 75% amino acid identity with SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243, at most 80% amino acid identity with SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243, at most 85% amino acid identity with SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243, at most 90% amino acid identity with SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243 or at most 95% amino acid identity with SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243.

[0194] In other aspects of this embodiment, a respirovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243. In other aspects of this embodiment, a respirovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243. In yet other aspects of this embodiment, a respirovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243. In other aspects of this embodiment, a respirovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243. In still other aspects of this embodiment, a respirovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243. In other aspects of this embodiment, a respirovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243.

[0195] In other aspects of this embodiment, a respirovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ

ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243. In other aspects of this embodiment, a respirovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243. In yet other aspects of this embodiment, a respirovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243. In other aspects of this embodiment, a respirovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243. In still other aspects of this embodiment, a respirovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243. In other aspects of this embodiment, a respirovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 or SEQ ID NO: 243.

[0196] In another embodiment, a Clostridial toxin translocation facilitating domain comprises a morbillivirus fusogenic peptide domain. In another aspect of this embodiment, a morbillivirus fusogenic peptide domain comprises a naturally occurring morbillivirus fusogenic peptide domain variant, such as, *e.g.*, a morbillivirus fusogenic peptide domain isoform or a morbillivirus fusogenic peptide domain subtype. In another aspect of this embodiment, a morbillivirus fusogenic peptide domain comprises a naturally occurring morbillivirus fusogenic peptide domain variant of SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253, such as, *e.g.*, a morbillivirus fusogenic peptide domain isoform of SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253 or a morbillivirus fusogenic peptide domain subtype of SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253. In still another aspect of this embodiment, a morbillivirus fusogenic peptide domain comprises a non-naturally occurring morbillivirus fusogenic peptide domain variant, such as, *e.g.*, a conservative morbillivirus fusogenic peptide domain variant, a non-conservative morbillivirus fusogenic peptide domain variant, a morbillivirus fusogenic peptide domain chimeric, an active morbillivirus fusogenic peptide domain fragment, or any combination thereof. In still another aspect of this embodiment, a morbillivirus fusogenic peptide domain comprises amino acids a non-naturally occurring morbillivirus fusogenic peptide domain variant of SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253,

such as, *e.g.*, a conservative morbillivirus fusogenic peptide domain variant of SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253, a non-conservative morbillivirus fusogenic peptide domain variant of SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253, an active morbillivirus fusogenic peptide domain fragment of SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253, or any combination thereof.

[0197] In other aspects of this embodiment, a morbillivirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253, at least 75% amino acid identity with SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253, at least 80% amino acid identity with SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253, at least 85% amino acid identity with SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253, at least 90% amino acid identity with SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253 or at least 95% amino acid identity with SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253. In yet other aspects of this embodiment, a morbillivirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253, at most 75% amino acid identity with SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253, at most 80% amino acid identity with SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253, at most 85% amino acid identity with SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253, at most 90% amino acid identity with SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253 or at most 95% amino acid identity with SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253.

[0198] In other aspects of this embodiment, a morbillivirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253. In other aspects of this embodiment, a morbillivirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253. In yet other aspects of this embodiment, a morbillivirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253. In other aspects of this embodiment, a morbillivirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253. In still other aspects of this embodiment, a morbillivirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253. In other aspects of this embodiment, a morbillivirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253.

[0199] In other aspects of this embodiment, a morbillivirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253. In other aspects of this embodiment, a morbillivirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253. In yet other aspects of this embodiment, a morbillivirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253. In other aspects of this embodiment, a morbillivirus fusogenic peptide domain comprises a polypeptide having,

e.g., at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253. In still other aspects of this embodiment, a morbillivirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253. In other aspects of this embodiment, a morbillivirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 or SEQ ID NO: 253.

[0200] In another embodiment, a Clostridial toxin translocation facilitating domain comprises an avulavirus fusogenic peptide domain. In another aspect of this embodiment, an avulavirus fusogenic peptide domain comprises a naturally occurring avulavirus fusogenic peptide domain variant, such as, *e.g.*, an avulavirus fusogenic peptide domain isoform or an avulavirus fusogenic peptide domain subtype. In another aspect of this embodiment, an avulavirus fusogenic peptide domain comprises a naturally occurring avulavirus fusogenic peptide domain variant of SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266, such as, *e.g.*, an avulavirus fusogenic peptide domain isoform of SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266 or an avulavirus fusogenic peptide domain subtype of SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266. In still another aspect of this embodiment, an avulavirus fusogenic peptide domain comprises a non-naturally occurring avulavirus fusogenic peptide domain variant, such as, *e.g.*, a conservative avulavirus fusogenic peptide domain variant, a non-conservative avulavirus fusogenic peptide domain variant, an avulavirus fusogenic peptide domain chimeric, an active avulavirus fusogenic peptide domain fragment, or any combination thereof. In still another aspect of this embodiment, an avulavirus fusogenic peptide domain comprises amino acids a non-naturally occurring avulavirus fusogenic peptide domain variant of SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266, such as, *e.g.*, a conservative avulavirus fusogenic peptide domain variant of SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266, a non-conservative avulavirus fusogenic peptide domain variant of SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261,

SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266, an active avulavirus fusogenic peptide domain fragment of SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266, or any combination thereof.

[0201] In other aspects of this embodiment, an avulavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266, at least 75% amino acid identity with SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266, at least 80% amino acid identity with SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266, at least 85% amino acid identity with SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266, at least 90% amino acid identity with SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266 or at least 95% amino acid identity with SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266. In yet other aspects of this embodiment, an avulavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266, at most 75% amino acid identity with SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266, at most 80% amino acid identity with SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266, at most 85% amino acid identity with SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266, at most 90% amino acid identity with SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266 or at most 95% amino acid identity with SEQ ID NO: 254, SEQ ID NO: 255,

SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266.

[0202] In other aspects of this embodiment, an avulavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266. In other aspects of this embodiment, an avulavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266. In yet other aspects of this embodiment, an avulavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266. In other aspects of this embodiment, an avulavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266. In still other aspects of this embodiment, an avulavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266. In other aspects of this embodiment, an avulavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266.

[0203] In other aspects of this embodiment, an avulavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266. In other aspects of this embodiment, an avulavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 254, SEQ ID

NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266. In yet other aspects of this embodiment, an avulavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266. In other aspects of this embodiment, an avulavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266. In still other aspects of this embodiment, an avulavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266. In other aspects of this embodiment, an avulavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 or SEQ ID NO: 266.

[0204] In another embodiment, a Clostridial toxin translocation facilitating domain comprises a henipavirus fusogenic peptide domain. In another aspect of this embodiment, a henipavirus fusogenic peptide domain comprises a naturally occurring henipavirus fusogenic peptide domain variant, such as, *e.g.*, a henipavirus fusogenic peptide domain isoform or a henipavirus fusogenic peptide domain subtype. In another aspect of this embodiment, a henipavirus fusogenic peptide domain comprises a naturally occurring henipavirus fusogenic peptide domain variant of SEQ ID NO: 267 or SEQ ID NO: 268, such as, *e.g.*, a henipavirus fusogenic peptide domain isoform of SEQ ID NO: 267 or SEQ ID NO: 268 or a henipavirus fusogenic peptide domain subtype of SEQ ID NO: 267 or SEQ ID NO: 268. In still another aspect of this embodiment, a henipavirus fusogenic peptide domain comprises a non-naturally occurring henipavirus fusogenic peptide domain variant, such as, *e.g.*, a conservative henipavirus fusogenic peptide domain variant, a non-conservative henipavirus fusogenic peptide domain variant, a henipavirus fusogenic peptide domain chimeric, an active henipavirus fusogenic peptide domain fragment, or any combination thereof. In still another aspect of this embodiment, a henipavirus fusogenic peptide domain comprises amino acids a non-naturally occurring henipavirus fusogenic peptide domain variant of SEQ ID NO: 267 or SEQ ID NO: 268, such as, *e.g.*, a conservative henipavirus fusogenic peptide domain variant of SEQ ID NO: 267 or SEQ ID NO: 268, a non-conservative henipavirus fusogenic peptide domain variant of SEQ ID NO: 267 or SEQ ID NO: 268, an active henipavirus fusogenic peptide domain fragment of SEQ ID NO: 267 or SEQ ID NO: 268, or any combination thereof.

[0205] In other aspects of this embodiment, a henipavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 267 or SEQ ID NO: 268, at least 75% amino acid identity with SEQ ID NO: 267 or SEQ ID NO: 268, at least 80% amino acid identity with SEQ ID NO: 267 or SEQ ID NO: 268, at least 85% amino acid identity with SEQ ID NO: 267 or SEQ ID NO: 268, at least 90% amino acid identity with SEQ ID NO: 267 or SEQ ID NO: 268 or at least 95% amino acid identity with SEQ ID NO: 267 or SEQ ID NO: 268. In yet other aspects of this embodiment, a henipavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 267 or SEQ ID NO: 268, at most 75% amino acid identity with SEQ ID NO: 267 or SEQ ID NO: 268, at most 80% amino acid identity with SEQ ID NO: 267 or SEQ ID NO: 268, at most 85% amino acid identity with SEQ ID NO: 267 or SEQ ID NO: 268, at most 90% amino acid identity with SEQ ID NO: 267 or SEQ ID NO: 268 or at most 95% amino acid identity with SEQ ID NO: 267 or SEQ ID NO: 268.

[0206] In other aspects of this embodiment, a henipavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 267 or SEQ ID NO: 268. In other aspects of this embodiment, a henipavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 267 or SEQ ID NO: 268. In yet other aspects of this embodiment, a henipavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 267 or SEQ ID NO: 268. In other aspects of this embodiment, a henipavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 267 or SEQ ID NO: 268. In still other aspects of this embodiment, a henipavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 267 or SEQ ID NO: 268. In other aspects of this embodiment, a henipavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 267 or SEQ ID NO: 268.

[0207] In other aspects of this embodiment, a henipavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 267 or SEQ ID NO: 268. In other aspects of this embodiment, a henipavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 267 or SEQ ID NO: 268. In yet other aspects of this embodiment, a henipavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 267 or SEQ ID NO: 268. In other aspects of this

embodiment, a henipavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 267 or SEQ ID NO: 268. In still other aspects of this embodiment, a henipavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 267 or SEQ ID NO: 268. In other aspects of this embodiment, a henipavirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 267 or SEQ ID NO: 268.

[0208] In another embodiment, a Clostridial toxin translocation facilitating domain comprises a metapneumovirus fusogenic peptide domain. In another aspect of this embodiment, a metapneumovirus fusogenic peptide domain comprises a naturally occurring metapneumovirus fusogenic peptide domain variant, such as, *e.g.*, a metapneumovirus fusogenic peptide domain isoform or a metapneumovirus fusogenic peptide domain subtype. In another aspect of this embodiment, a metapneumovirus fusogenic peptide domain comprises a naturally occurring metapneumovirus fusogenic peptide domain variant of SEQ ID NO: 269, such as, *e.g.*, a metapneumovirus fusogenic peptide domain isoform of SEQ ID NO: 269 or a metapneumovirus fusogenic peptide domain subtype of SEQ ID NO: 269. In still another aspect of this embodiment, a metapneumovirus fusogenic peptide domain comprises a non-naturally occurring metapneumovirus fusogenic peptide domain variant, such as, *e.g.*, a conservative metapneumovirus fusogenic peptide domain variant, a non-conservative metapneumovirus fusogenic peptide domain variant, a metapneumovirus fusogenic peptide domain chimeric, an active metapneumovirus fusogenic peptide domain fragment, or any combination thereof. In still another aspect of this embodiment, a metapneumovirus fusogenic peptide domain comprises amino acids a non-naturally occurring metapneumovirus fusogenic peptide domain variant of SEQ ID NO: 269, such as, *e.g.*, a conservative metapneumovirus fusogenic peptide domain variant of SEQ ID NO: 269, a non-conservative metapneumovirus fusogenic peptide domain variant of SEQ ID NO: 269, an active metapneumovirus fusogenic peptide domain fragment of SEQ ID NO: 269, or any combination thereof.

[0209] In other aspects of this embodiment, a metapneumovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 269, at least 75% amino acid identity with SEQ ID NO: 269, at least 80% amino acid identity with SEQ ID NO: 269, at least 85% amino acid identity with SEQ ID NO: 269, at least 90% amino acid identity with SEQ ID NO: 269 or at least 95% amino acid identity with SEQ ID NO: 269. In yet other aspects of this embodiment, a metapneumovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 269, at most 75% amino acid identity with SEQ ID NO: 269, at most 80% amino acid identity with SEQ ID NO: 269, at most 85% amino acid identity with SEQ ID NO: 269, at most 90% amino acid identity with SEQ ID NO: 269 or at most 95% amino acid identity with SEQ ID NO: 269.

[0210] In other aspects of this embodiment, a metapneumovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 269. In other aspects of this embodiment, a metapneumovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 269. In yet other aspects of this embodiment, a metapneumovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 269. In other aspects of this embodiment, a metapneumovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 269. In still other aspects of this embodiment, a metapneumovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 269. In other aspects of this embodiment, a metapneumovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 non-contiguous amino acid additions relative to SEQ ID NO: 269.

[0211] In other aspects of this embodiment, a metapneumovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 269. In other aspects of this embodiment, a metapneumovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid substitutions relative to SEQ ID NO: 269. In yet other aspects of this embodiment, a metapneumovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 269. In other aspects of this embodiment, a metapneumovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid deletions relative to SEQ ID NO: 269. In still other aspects of this embodiment, a metapneumovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 269. In other aspects of this embodiment, a metapneumovirus fusogenic peptide domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or 10 contiguous amino acid additions relative to SEQ ID NO: 269.

[0212] Aspects of the present invention provide, in part, an altered targeting domain. As used herein, the term "altered targeting domain" means any polypeptide that can selectively bind to a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell and initiate the overall internalization mechanism whereby the modified Clostridial toxin disclosed in the present specification intoxicates a target cell. As used herein, the term "selectively" means having a highly preferred activity or effect. As used herein, the term "selectively bind" means a molecule is able to bind its target receptor under

physiological conditions, or in vitro conditions substantially approximating physiological conditions, to a statistically significantly greater degree relative to other, non-target receptors. Thus, with reference to an altered targeting domain of the present specification, there is a discriminatory binding of the altered targeting domain to a non-Clostridial toxin receptor presence in a non-Clostridial toxin target cell.

[0213] An altered targeting domain disclosed in the present specification facilitates the binding activity of the modified Clostridial toxins disclosed in the present specification to a non-Clostridial toxin receptor located at the surface of a Clostridial toxin target cell. As used herein, the term "binding activity" means that one molecule is directly or indirectly contacting another molecule via at least one intermolecular or intramolecular force, including, without limitation, a covalent bond, an ionic bond, a metallic bond, a hydrogen bond, a hydrophobic interaction, a van der Waals interaction, and the like, or any combination thereof. "Bound" and "bind" are considered terms for binding.

[0214] As used herein, the term "binding affinity" means how strong a molecule's binding activity is for a particular receptor. In general, high binding affinity results from greater intermolecular force between a binding domain and its receptor while low binding affinity involves less intermolecular force between the ligand and its receptor. High binding affinity involves a longer residence time for the binding domain at its receptor binding site than is the case for low binding affinity. As such, a molecule with a high binding affinity means a lower concentration of that molecule is required to maximally occupy the binding sites of a receptor and trigger a physiological response. Conversely, low binding affinity means a relatively high concentration of a molecule is required before the receptor binding sites of a receptor is maximally occupied and the maximum physiological response is achieved. Thus, modified Clostridial toxins with increased binding activity due to high binding affinity will allow administration of reduced doses of the toxin, thereby reducing or preventing unwanted side-effects associated with toxin dispersal into non-targeted areas.

[0215] As used herein, the term "binding specificity" means how specific a molecule's binding activity is one particular receptor. In general, high binding specificity results in a more exclusive interaction with one particular receptor or subgroup of receptors while low binding specificity results in a more promiscuous interaction with a larger group of receptors. As such, a molecule with a high binding specificity means that molecule will occupy the binding sites of a particular receptor and trigger a physiological response. Conversely, low binding specificity means a molecule will occupy the binding sites of a many receptors and trigger a multitude of physiological responses. Thus, modified Clostridial toxins with increased binding activity due to high binding specificity will only target non-Clostridial toxin receptors present on a subgroup of non-Clostridial toxin target cells, thereby reducing the side effects associated with the targeting of all non-Clostridial toxin target cells.

[0216] In addition to its altered targeting activity, replacement of a naturally-occurring targeting domain with an altered target domain disclosed in the specification has an added advantage of reducing the

likelihood of the modified toxin from eliciting an immunogenic response. Regions found in the H_{CC} targeting domain are bound by neutralizing anti-BoNT/A antibodies, see, *e.g.*, M. Zouhair Atassi et al., *Mapping of the Antibody-binding Regions on Botulinum Neurotoxin H-chain Domain 855–1296 with Anti-toxin Antibodies from Three Host Species*, 15 J. PROT. CHEM. 691-700, (1996); M. Zouhair Atassi & Behzod Z. Dolimbek, *Mapping of the Antibody-binding Profile on Botulinum Neurotoxin A H_N-domain (residues 449–859) with Anti-toxin Antibodies from Four Host Species. Antigenic Profile of the Entire H-chain of Botulinum Neurotoxin A*, 23(1) PROTEIN J. 39-52, (2004). Therefore, elimination of this targeting domain will reduce the likelihood of an immunogenic response because 1) the Clostridial toxin H_{CC} targeting domain is absent; 2) an altered targeting domain derived from a human will most likely not elicit an immunogenic response in a patient because it is a human polypeptide.

[0217] As used herein, the term "non-Clostridial toxin target cell" means a cell that is not a naturally occurring cell that a naturally occurring Clostridial toxin is capable of intoxicating, including, without limitation, sensory neurons; autonomic neurons, such as, *e.g.*, sympathetic neurons and parasympathetic neurons; and non-neuronal cells, such as, *e.g.*, anterior pituitary cells; adrenal cells, such as. *e.g.*, chromaffin cells of the adrenal medulla; pancreatic cells, such as. *e.g.*, pancreatic acinar cells, pancreatic islet β cells; ovarian cells; kidney cells, such as. *e.g.*, inner medullary collecting duct (IMCD) cells; stomach cells, such as, *e.g.*, enterochromaffin cells; blood cells, such as. *e.g.*, eurythrocytes, leucocytes, platelets, neutrophils, eosinophils, mast cells; epithelial cells, such as. *e.g.*, those of the apical plasma membrane; fibroblasts; thyroid cells; chondrocytes; muscle cells; hepatocytes; glandular cells such as, *e.g.*, pituitary cells, chromaffin cells.

[0218] It is envisioned that any and all altered targeting domains that exhibits a binding activity for a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell can be used to practice aspects of the present invention, including, without limitation, polypeptides that selectively bind to a receptor present on a sensory neuron, an autonomic neuron or a non-neuronal cell. Polypeptides useful as altered targeting domains useful to practice aspect of the present invention include, without limitation, an opioid peptide, such as, *e.g.*, an enkephalin, a bovine adrenomedullary-22 (BAM22) peptide, an endomorphin, an endorphin, a dynorphin, a nociceptin or a hemorphin; a melanocortin peptide, such as, *e.g.*, an α-melanocyte stimulating hormones (α-MSH), a β-melanocyte stimulating hormones (β-MSH), a γ-melanocyte stimulating hormones (γ-MSH), an adrenocorticotropin (ACTH), a Corticotropin-like intermediary peptide (CLIP), a β-lipotropin (β-LPH) and a γ-lipotropin (γ-LPH); a galanin, such as, *e.g.*, a galanin and a galanin message-associated peptide (GMAP); a granin, such as, *e.g.*, a chromogranin A peptide like a β-granin, a vasostatin, a chromostatin, a pancreastatin, a WE-14, a catestatin, a parastatin and a GE-25, a chromogranin B (secretogranin I) peptide like a GAWK peptide, an adrenomedullary peptide and a secretolytin and a chromogranin C (secretogranin II) peptide like secretoneurin, EM66 and manserin; a tachykinin peptide, such as, *e.g.*, Substance P, neuropeptide K (NPK), neuropeptide gamma (NP gamma), neurokinin A (NKA; Substance K, neurokinin alpha, neuromedin L), neurokinin B (NKB), a hemokinin and a endokinin; a cholecystokinin, such as, *e.g.*, a cholecystokinin 58, a cholecystokinin 39,

a cholecystokinin 33, a cholecystokinin 12 and a cholecystokinin 8; a Neuropeptide Y related peptide, such as, *e.g.*, a Neuropeptide Y (NPY), a Peptide YY (PYY), Pancreatic peptide (PP) and a Pancreatic icosapeptide (PIP); , a kinin peptide, such as, *e.g.*, a bradykinin, a kallidan, a desArg^{9} bradykinin and a desArg^{10} bradykinin; a protease activated receptor (PAR) peptide, such as, *e.g.*, a PAR1 peptide, a PAR2 peptide, a PAR3 peptide and a PAR4 peptide; a corticotropin-releasing hormone; a thyrotropin-releasing hormone; a somatostatin; a leukemia inhibitor factor (LIF); and an interleukin-1 (IL1).

[0219] An altered targeting domain includes, without limitation, naturally occurring altered targeting domain variants, such as, *e.g.*, altered targeting domain isoforms; non-naturally occurring altered targeting domain variants, such as, *e.g.*, conservative altered targeting domain variants, non-conservative altered targeting domain variants, altered targeting domain chimerics, active altered targeting domain fragments thereof, or any combination thereof.

[0220] As used herein, the term "variant," when used to describe an altered targeting domain variant, whether naturally-occurring or non-naturally-occurring, means an altered targeting domain that has at least one amino acid change from the corresponding region of the disclosed reference sequences and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, all altered targeting domain variants disclosed in the present specification are capable of selectively binding to a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell and initiate the overall internalization mechanism whereby a modified Clostridial toxin disclosed in the present specification intoxicates a non-Clostridial toxin target cell. As non-limiting examples, an endorphin-β variant derived from SEQ ID NO: 17 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 17; a Dymorphin A variant derived from SEQ ID NO: 21 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 21; a nociceptin variant derived from SEQ ID NO: 52 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 52; a galanin variant derived from SEQ ID NO: 72 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 72; an adrenomedullary peptide variant derived from SEQ ID NO: 83 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 83; a Substance P variant derived from SEQ ID NO: 88 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 88; an endokinin variant derived from SEQ ID NO: 97 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 97; a CCK variant derived from SEQ ID NO: 100 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 100; a NPY variant derived from SEQ ID NO: 116 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 116; and a PP variant derived from SEQ ID NO: 118 will have at

least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to SEQ ID NO: 118.

[0221] It is recognized by those of skill in the art that there can be naturally occurring altered targeting domain variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. As used herein, the term "naturally occurring altered targeting domain variant" means any altered targeting domain produced by a naturally-occurring process, including, without limitation, altered targeting domain isoforms produced from alternatively-spliced transcripts, altered targeting domain isoforms produced by spontaneous mutation and altered targeting domain subtypes. A naturally occurring altered targeting domain variant can function in substantially the same manner as the reference altered targeting domain on which the naturally occurring altered targeting domain variant is based, and can be substituted for the reference altered targeting domain in any aspect of the present invention. A naturally occurring altered targeting domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids or 100 or more amino acids from the reference altered targeting domain on which the naturally occurring altered targeting domain variant is based. A naturally occurring altered targeting domain variant can also substitute, *e.g.*, at least 2 contiguous amino acids, at least 3 contiguous amino acids, at least 4 contiguous amino acids, at least 5 contiguous amino acids, at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference altered targeting domain on which the naturally occurring altered targeting domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference altered targeting domain on which the naturally occurring altered targeting domain variant is based.

[0222] A non-limiting example of a naturally occurring altered targeting domain variant is an altered targeting domain isoform such as, *e.g.*, an opioid peptide, a melanocortin peptide, a galanin, a granin, a tachykinin peptide, a cholecystokinin, a Neuropeptide Y related peptide, a kinin peptide, a protease activated receptor (PAR) peptide, a corticotropin-releasing hormone, a thyrotropin-releasing hormone and somatostatin. An altered targeting domain isoform can function in substantially the same manner as the reference altered targeting domain on which the altered targeting domain isoform is based, and can be substituted for the reference altered targeting domain in any aspect of the present invention.

[0223] As used herein, the term "non-naturally occurring altered targeting domain variant" means any altered targeting domain produced with the aid of human manipulation, including, without limitation, altered targeting domains produced by genetic engineering using random mutagenesis or rational design and altered targeting domains produced by chemical synthesis. Non-limiting examples of non-naturally occurring altered targeting domain variants include, *e.g.*, conservative altered targeting domain variants,

non-conservative altered targeting domain variants, altered targeting domain chimeric variants and active altered targeting domain fragments.

[0224] As used herein, the term "conservative altered targeting domain variant" means an altered targeting domain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference altered targeting domain sequence. Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative altered targeting domain variant can function in substantially the same manner as the reference altered targeting domain on which the conservative altered targeting domain variant is based, and can be substituted for the reference altered targeting domain in any aspect of the present invention. A conservative altered targeting domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the reference altered targeting domain on which the conservative altered targeting domain variant is based. A conservative altered targeting domain variant can also substitute, *e.g.*, at least 2 contiguous amino acids, at least 3 contiguous amino acids, at least 4 contiguous amino acids, at least 5 contiguous amino acids, at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference altered targeting domain on which the conservative altered targeting domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference altered targeting domain on which the conservative altered targeting domain variant is based. Non-limiting examples of a conservative altered targeting domain variant include, *e.g.*, conservative opioid peptide variants, conservative melanocortin peptide variants, conservative galanin variants, conservative granin variants, conservative tachykinin peptide variants, conservative cholecystokinin variants, conservative Neuropeptide Y related peptide variants, conservative kinin peptide variants, conservative PAR peptide variants, conservative corticotropin-releasing hormone variants, conservative thyrotropin-releasing hormone variants and conservative somatostatin variants.

[0225] As used herein, the term "non-conservative altered targeting domain variant" means an altered targeting domain in which 1) at least one amino acid is deleted from the reference altered targeting domain on which the non-conservative altered targeting domain variant is based; 2) at least one amino acid added to the reference altered targeting domain on which the non-conservative altered targeting domain is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference altered targeting domain sequence. A non-conservative altered targeting domain variant can function in substantially the same manner as the reference altered targeting domain on which the non-conservative

altered targeting domain variant is based, and can be substituted for the reference altered targeting domain in any aspect of the present invention. A non-conservative altered targeting domain variant can delete one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids from the reference altered targeting domain on which the non-conservative altered targeting domain variant is based. A non-conservative altered targeting domain variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids to the reference altered targeting domain on which the non-conservative altered targeting domain variant is based. A non-conservative altered targeting domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the reference altered targeting domain on which the non-conservative altered targeting domain variant is based. A non-conservative altered targeting domain variant can also substitute, *e.g.*, at least 2 contiguous amino acids, at least 3 contiguous amino acids, at least 4 contiguous amino acids, at least 5 contiguous amino acids, at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference altered targeting domain on which the non-conservative altered targeting domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference altered targeting domain on which the non-conservative altered targeting domain variant is based. Non-limiting examples of a non-conservative altered targeting domain variant include, *e.g.*, non-conservative opioid peptide variants, non-conservative melanocortin peptide variants, non-conservative galanin variants, non-conservative granin variants, non-conservative tachykinin peptide variants, non-conservative cholecystokinin variants, non-conservative Neuropeptide Y related peptide variants, non-conservative kinin peptide variants, non-conservative PAR peptide variants, non-conservative corticotropin-releasing hormone variants, non-conservative thyrotropin-releasing hormone variants and non-conservative somatostatin variants.

[0226] As used herein, the term "altered targeting domain chimeric" means a polypeptide comprising at least a portion of an altered targeting domain and at least a portion of at least one other polypeptide to form an altered targeting domain with at least one property different from the reference altered targeting domain, with the proviso that this altered targeting domain chimeric is still capable of selectively binding to a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell and initiate the overall internalization mechanism whereby a modified Clostridial toxin intoxicates a target cell.

[0227] As used herein, the term "active altered targeting domain fragment" means any of a variety of altered targeting domain fragments can be useful in aspects of the present invention with the proviso that these active fragments are still capable of selectively binding to a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell and initiate the overall internalization mechanism whereby a Clostridial

toxin intoxicates a target cell. Thus, aspects of this embodiment can include altered targeting domains comprising a length of, *e.g.*, at least 5 amino acids, at least 10 amino acids, at least 20 amino acids, at least 30 amino acids, at least 40 amino acids, at least 50 amino acids, at least 100 amino acids, at least 150 amino acids, at least 200 amino acids, at least 250 amino acids, at least 300 amino acids, at least 350 amino acids, at least 400 amino acids and at least 450 amino acids. Other aspects of this embodiment can include altered targeting domains comprising a length of, *e.g.*, at most 5 amino acids, at most 10 amino acids, at most 20 amino acids, at most 30 amino acids, at most 40 amino acids, at most 50 amino acids, at most 100 amino acids, at most 150 amino acids, at most 200 amino acids, at most 250 amino acids, at most 300 amino acids, at most 350 amino acids, at most 400 amino acids and at most 450 amino acids.

[0228] Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring altered targeting domain variants and non-naturally-occurring altered targeting domain variants, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0229] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification comprises an altered targeting domain. In an aspect of this embodiment, an altered targeting domain comprises a naturally occurring altered targeting domain variant, such as, *e.g.*, an altered targeting domain isoform or an altered targeting domain subtype. In another aspect of this embodiment, a Clostridial toxin altered targeting domain comprises a non-naturally occurring altered targeting domain variant, such as, *e.g.*, a conservative altered targeting domain variant, a non-conservative altered targeting domain variant, an altered targeting domain chimeric, an active altered targeting domain fragment, or any combination thereof.

[0230] An example of an altered targeting domain disclosed in the present specification is, *e.g.*, a opioid peptide, such as, *e.g.*, an enkephalin, an endomorphin, an endorphin, a dynorphin, a nociceptin or a hemorphin. Thus, in an embodiment, an altered targeting domain is derived from an opioid peptide.

[0231] In another embodiment, an opioid peptide comprising an altered targeting domain is an enkephalin. In aspects of this embodiment, an enkephalin comprising an altered targeting domain is derived from a Leu-enkephalin, a Met-enkephalin, a Met-enkephalin MRGL or a Met-enkephalin MRF. In other aspects of this embodiment, an enkephalin comprising an altered targeting domain is SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12.

[0232] In other aspects of this embodiment, an enkephalin comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12, at least 75% amino acid identity with SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO:

12, at least 80% amino acid identity with SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12, at least 85% amino acid identity with SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12, at least 90% amino acid identity with SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12 or at least 95% amino acid identity with SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, an enkephalin comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12, at most 75% amino acid identity with SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12, at most 80% amino acid identity with SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12, at most 85% amino acid identity with SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12, at most 90% amino acid identity with SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12 or at most 95% amino acid identity with SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12.

[0233] In other aspects of this embodiment, an enkephalin comprising an altered targeting domain has, *e.g.*, at least one, two or three non-contiguous amino acid substitutions relative to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12. In other aspects of this embodiment, an enkephalin comprising an altered targeting domain has, *e.g.*, at most one, two or three non-contiguous amino acid substitutions relative to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, an enkephalin comprising an altered targeting domain has, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, an enkephalin comprising an altered targeting domain has, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12. In still other aspects of this embodiment, an enkephalin comprising an altered targeting domain has, *e.g.*, at least one, two or three non-contiguous amino acid additions relative to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, an enkephalin comprising an altered targeting domain has, *e.g.*, at most one, two or three non-contiguous amino acid additions relative to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12.

[0234] In other aspects of this embodiment, an enkephalin comprising an altered targeting domain has, *e.g.*, at least one, two or three contiguous amino acid substitutions relative to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12. In other aspects of this embodiment, an enkephalin comprising an altered targeting domain has, *e.g.*, at most one, two or three contiguous amino acid substitutions relative to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, an enkephalin comprising an altered targeting domain has, *e.g.*, at least one, two or three contiguous amino acid deletions relative to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, an enkephalin comprising an altered targeting domain has, *e.g.*, at most one, two or three contiguous amino acid deletions relative to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12. In still other aspects of this embodiment, an enkephalin

comprising an altered targeting domain has, *e.g.*, at least one, two or three contiguous amino acid additions relative to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, an enkephalin comprising an altered targeting domain has, *e.g.*, at most one, two or three contiguous amino acid additions relative to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12.

[0235] In another embodiment, an opioid peptide comprising an altered targeting domain is a bovine adrenomedullary-22 (BAM22) peptide. In aspects of this embodiment, a BAM22 peptide comprising an altered targeting domain is derived from a BAM22 peptide (1-12), a BAM22 peptide (6-22), a BAM22 peptide (8-22) or a BAM22 peptide (1-22). In other aspects of this embodiment, a BAM22 peptide comprising an altered targeting domain comprises amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177.

[0236] In other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177, at least 75% amino acid identity with amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177, at least 80% amino acid identity with amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177, at least 85% amino acid identity with amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173;

amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177, at least 90% amino acid identity with amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177 or at least 95% amino acid identity with amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177.

[0237] In yet other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177, at most 75% amino acid identity with amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177, at most 80% amino acid identity with amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177, at most 85% amino acid identity with amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-

22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177, at most 90% amino acid identity with amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177 or at most 95% amino acid identity with amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177.

[0238] In other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid substitutions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177. In other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid substitutions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177. In yet other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid deletions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of

SEQ ID NO: 177. In yet other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid deletions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177. In still other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid additions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177. In yet other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid additions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177.

[0239] In other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid substitutions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177. In other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid substitutions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177. In yet other aspects

of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid deletions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177. In yet other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid deletions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177. In still other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid additions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177. In yet other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid additions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177.

[0240] In another embodiment, an opioid peptide comprising an altered targeting domain is an endomorphin. In aspects of this embodiment, an endomorphin comprising an altered targeting domain is derived from an endomorphin-1 or an endomorphin-2. In other aspects of this embodiment, an endomorphin comprising an altered targeting domain is SEQ ID NO: 13 or SEQ ID NO: 14.

[0241] In other aspects of this embodiment, an endomorphin comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 13 or SEQ ID NO: 14, at least 75% amino

acid identity with SEQ ID NO: 13 or SEQ ID NO: 14, at least 80% amino acid identity with SEQ ID NO: 13 or SEQ ID NO: 14, at least 85% amino acid identity with SEQ ID NO: 13 or SEQ ID NO: 14, at least 90% amino acid identity with SEQ ID NO: 13 or SEQ ID NO: 14 or at least 95% amino acid identity with SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, an endomorphin comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 13 or SEQ ID NO: 14, at most 75% amino acid identity with SEQ ID NO: 13 or SEQ ID NO: 14, at most 80% amino acid identity with SEQ ID NO: 13 or SEQ ID NO: 14, at most 85% amino acid identity with SEQ ID NO: 13 or SEQ ID NO: 14, at most 90% amino acid identity with SEQ ID NO: 13 or SEQ ID NO: 14 or at most 95% amino acid identity with SEQ ID NO: 13 or SEQ ID NO: 14.

[0242] In other aspects of this embodiment, an endomorphin comprising an altered targeting domain has, *e.g.*, at least one, two or three non-contiguous amino acid substitutions relative to SEQ ID NO: 13 or SEQ ID NO: 14. In other aspects of this embodiment, an endomorphin comprising an altered targeting domain has, *e.g.*, at most one, two or three non-contiguous amino acid substitutions relative to SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, an endomorphin comprising an altered targeting domain has, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, an endomorphin comprising an altered targeting domain has, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 13 or SEQ ID NO: 14. In still other aspects of this embodiment, an endomorphin comprising an altered targeting domain has, *e.g.*, at least one, two or three non-contiguous amino acid additions relative to SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, an endomorphin comprising an altered targeting domain has, *e.g.*, at most one, two or three non-contiguous amino acid additions relative to SEQ ID NO: 13 or SEQ ID NO: 14.

[0243] In other aspects of this embodiment, an endomorphin comprising an altered targeting domain has, *e.g.*, at least one, two or three contiguous amino acid substitutions relative to SEQ ID NO: 13 or SEQ ID NO: 14. In other aspects of this embodiment, an endomorphin comprising an altered targeting domain has, *e.g.*, at most one, two or three contiguous amino acid substitutions relative to SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, an endomorphin comprising an altered targeting domain has, *e.g.*, at least one, two or three contiguous amino acid deletions relative to SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, an endomorphin comprising an altered targeting domain has, *e.g.*, at most one, two or three contiguous amino acid deletions relative to SEQ ID NO: 13 or SEQ ID NO: 14. In still other aspects of this embodiment, an endomorphin comprising an altered targeting domain has, *e.g.*, at least one, two or three contiguous amino acid additions relative to SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, an endomorphin comprising an altered targeting domain has, *e.g.*, at most one, two or three contiguous amino acid additions relative to SEQ ID NO: 13 or SEQ ID NO: 14.

[0244] In another embodiment, an opioid peptide comprising an altered targeting domain is an endorphin. In aspects of this embodiment, an endorphin comprising an altered targeting domain is derived from an endorphin-α, a neoendorphin-α, an endorphin-β, a neoendorphin-β or an endorphin-γ. In other aspects of this embodiment, an enkephalin comprising an altered targeting domain is SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20.

[0245] In other aspects of this embodiment, an endorphin comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20, at least 75% amino acid identity with SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20, at least 80% amino acid identity with SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20, at least 85% amino acid identity with SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20, at least 90% amino acid identity with SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20 or at least 95% amino acid identity with SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20. In yet other aspects of this embodiment, an endorphin comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20, at most 75% amino acid identity with SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20, at most 80% amino acid identity with SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20, at most 85% amino acid identity with SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20, at most 90% amino acid identity with SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20 or at most 95% amino acid identity with SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20.

[0246] In other aspects of this embodiment, an endorphin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid substitutions relative to SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20. In other aspects of this embodiment, an endorphin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid substitutions relative to SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20. In yet other aspects of this embodiment, an endorphin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid deletions relative to SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20. In yet other aspects of this embodiment, an endorphin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid deletions relative to SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20. In still other aspects of this embodiment, an endorphin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid

additions relative to SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20. In yet other aspects of this embodiment, an endorphin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid additions relative to SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20.

[0247] In other aspects of this embodiment, an endorphin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid substitutions relative to SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20. In other aspects of this embodiment, an endorphin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid substitutions relative to SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20. In yet other aspects of this embodiment, an endorphin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid deletions relative to SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20. In yet other aspects of this embodiment, an endorphin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid deletions relative to SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20. In still other aspects of this embodiment, an endorphin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid additions relative to SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20. In yet other aspects of this embodiment, an endorphin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid additions relative to SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20.

[0248] In another embodiment, an opioid peptide comprising an altered targeting domain is a dynorphin. In aspects of this embodiment, a dynorphin comprising an altered targeting domain is derived from a dynorphin A, a dynorphin B (leumorphin) or a rimorphin. In other aspects of this embodiment, a dynorphin comprising an altered targeting domain is SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50 or SEQ ID NO: 51.

[0249] In other aspects of this embodiment, a dynorphin comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46, at least 75% amino acid identity with SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46, at least 80% amino acid identity with SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46, at least 85% amino acid identity with SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46, at least 90% amino acid identity with SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46 or at least 95% amino acid identity with SEQ ID NO: 21, SEQ ID NO:

30 or SEQ ID NO: 46. In yet other aspects of this embodiment, a dynorphin comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46, at most 75% amino acid identity with SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46, at most 80% amino acid identity with SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46, at most 85% amino acid identity with SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46, at most 90% amino acid identity with SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46 or at most 95% amino acid identity with SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46.

[0250] In other aspects of this embodiment, a dynorphin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46. In other aspects of this embodiment, a dynorphin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46. In yet other aspects of this embodiment, a dynorphin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46. In yet other aspects of this embodiment, a dynorphin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46. In still other aspects of this embodiment, a dynorphin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46. In yet other aspects of this embodiment, a dynorphin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46.

[0251] In other aspects of this embodiment, a dynorphin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46. In other aspects of this embodiment, a dynorphin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46. In yet other aspects of this embodiment, a dynorphin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46. In yet other aspects of this embodiment, a dynorphin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46. In still other aspects of this embodiment, a dynorphin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46. In yet other aspects of this embodiment, a dynorphin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven,

eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 21, SEQ ID NO: 30 or SEQ ID NO: 46.

[0252] In another embodiment, an opioid peptide comprising an altered targeting domain is a nociceptin. In aspects of this embodiment, a nociceptin comprising an altered targeting domain is derived from a nociceptin RK, a nociceptin, a neuropeptide 1, a neuropeptide 2 or a neuropeptide 3. In other aspects of this embodiment, a nociceptin comprising an altered targeting domain is SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61.

[0253] In other aspects of this embodiment, a nociceptin comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61, at least 75% amino acid identity with SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61, at least 80% amino acid identity with SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61, at least 85% amino acid identity with SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61, at least 90% amino acid identity with SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61 or at least 95% amino acid identity with SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61. In yet other aspects of this embodiment, a nociceptin comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61, at most 75% amino acid identity with SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61, at most 80% amino acid identity with SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61, at most 85% amino acid identity with SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61, at most 90% amino acid identity with SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61 or at most 95% amino acid identity with SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61.

[0254] In other aspects of this embodiment, a nociceptin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61. In other aspects of this embodiment, a nociceptin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61. In yet other aspects of this embodiment, a nociceptin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61. In yet other aspects of this embodiment, a nociceptin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61. In still other aspects of this embodiment, a nociceptin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino

acid additions relative to SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61. In yet other aspects of this embodiment, a nociceptin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61.

[0255] In other aspects of this embodiment, a nociceptin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61. In other aspects of this embodiment, a nociceptin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61. In yet other aspects of this embodiment, a nociceptin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61. In yet other aspects of this embodiment, a nociceptin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61. In still other aspects of this embodiment, a nociceptin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61. In yet other aspects of this embodiment, a nociceptin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 52, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61.

[0256] Another example of an altered targeting domain disclosed in the present specification is, *e.g.*, a melanocortin peptide, such as, *e.g.*, a melanocyte stimulating hormone, an adrenocorticotropin, a Corticotropin-like intermediary peptide) or a lipotropin. Thus, in an embodiment, an altered targeting domain is derived from a melanocortin peptide.

[0257] In another embodiment, a melanocortin peptide comprising an altered targeting domain is a melanocyte stimulating hormone. In aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain is derived from an α-melanocyte stimulating hormones (α-MSH), a β-melanocyte stimulating hormones (β-MSH), a γ-melanocyte stimulating hormones (γ-MSH). In other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain is SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64.

[0258] In other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64, at least 75% amino acid identity with SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64, at least 80% amino acid identity with SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64, at least 85%

amino acid identity with SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64, at least 90% amino acid identity with SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64 or at least 95% amino acid identity with SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64. In yet other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64, at most 75% amino acid identity with SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64, at most 80% amino acid identity with SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64, at most 85% amino acid identity with SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64, at most 90% amino acid identity with SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64 or at most 95% amino acid identity with SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64.

[0259] In other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid substitutions relative to SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64. In other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid substitutions relative to SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64. In yet other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid deletions relative to SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64. In yet other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid deletions relative to SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64. In still other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid additions relative to SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64. In yet other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid additions relative to SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64.

[0260] In other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid substitutions relative to SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64. In other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid substitutions relative to SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64. In yet other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid deletions relative to SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64. In yet other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid deletions relative to SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64. In still other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid additions

relative to SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64. In yet other aspects of this embodiment, a melanocyte stimulating hormone comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid additions relative to SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64.

[0261] In another embodiment, a melanocortin peptide comprising an altered targeting domain is an adrenocorticotropin. In aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain is derived from an adrenocorticotropin (ACTH) or a Corticotropin-like intermediary peptide (CLIP). In other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain is SEQ ID NO: 65 or SEQ ID NO: 66.

[0262] In other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 65 or SEQ ID NO: 66, at least 75% amino acid identity with SEQ ID NO: 65 or SEQ ID NO: 66, at least 80% amino acid identity with SEQ ID NO: 65 or SEQ ID NO: 66, at least 85% amino acid identity with SEQ ID NO: 65 or SEQ ID NO: 66, at least 90% amino acid identity with SEQ ID NO: 65 or SEQ ID NO: 66 or at least 95% amino acid identity with SEQ ID NO: 65 or SEQ ID NO: 66. In yet other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 65 or SEQ ID NO: 66, at most 75% amino acid identity with SEQ ID NO: 65 or SEQ ID NO: 66, at most 80% amino acid identity with SEQ ID NO: 65 or SEQ ID NO: 66, at most 85% amino acid identity with SEQ ID NO: 65 or SEQ ID NO: 66, at most 90% amino acid identity with SEQ ID NO: 65 or SEQ ID NO: 66 or at most 95% amino acid identity with SEQ ID NO: 65 or SEQ ID NO: 66.

[0263] In other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid substitutions relative to SEQ ID NO: 65 or SEQ ID NO: 66. In other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid substitutions relative to SEQ ID NO: 65 or SEQ ID NO: 66. In yet other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid deletions relative to SEQ ID NO: 65 or SEQ ID NO: 66. In yet other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid deletions relative to SEQ ID NO: 65 or SEQ ID NO: 66. In still other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid additions relative to SEQ ID NO: 65 or SEQ ID NO: 66. In yet other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid additions relative to SEQ ID NO: 65 or SEQ ID NO: 66.

[0264] In other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid substitutions relative to SEQ ID NO: 65 or SEQ ID NO: 66. In other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid substitutions relative to SEQ ID NO: 65 or SEQ ID NO: 66. In yet other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid deletions relative to SEQ ID NO: 65 or SEQ ID NO: 66. In yet other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid deletions relative to SEQ ID NO: 65 or SEQ ID NO: 66. In still other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid additions relative to SEQ ID NO: 65 or SEQ ID NO: 66. In yet other aspects of this embodiment, an adrenocorticotropin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid additions relative to SEQ ID NO: 65 or SEQ ID NO: 66.

[0265] In another embodiment, a melanocortin peptide comprising an altered targeting domain is a lipotropin. In aspects of this embodiment, a lipotropin comprising an altered targeting domain is derived from a β-lipotropin (β-LPH) or a γ-lipotropin (γ-LPH). In other aspects of this embodiment, a lipotropin comprising an altered targeting domain is SEQ ID NO: 67 or SEQ ID NO: 68.

[0266] In other aspects of this embodiment, a lipotropin comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 67 or SEQ ID NO: 68, at least 75% amino acid identity with SEQ ID NO: 67 or SEQ ID NO: 68, at least 80% amino acid identity with SEQ ID NO: 67 or SEQ ID NO: 68, at least 85% amino acid identity with SEQ ID NO: 67 or SEQ ID NO: 68, at least 90% amino acid identity with SEQ ID NO: 67 or SEQ ID NO: 68 or at least 95% amino acid identity with SEQ ID NO: 67 or SEQ ID NO: 68. In yet other aspects of this embodiment, a lipotropin comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 67 or SEQ ID NO: 68, at most 75% amino acid identity with SEQ ID NO: 67 or SEQ ID NO: 68, at most 80% amino acid identity with SEQ ID NO: 67 or SEQ ID NO: 68, at most 85% amino acid identity with SEQ ID NO: 67 or SEQ ID NO: 68, at most 90% amino acid identity with SEQ ID NO: 67 or SEQ ID NO: 68 or at most 95% amino acid identity with SEQ ID NO: 67 or SEQ ID NO: 68.

[0267] In other aspects of this embodiment, a lipotropin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 67 or SEQ ID NO: 68. In other aspects of this embodiment, a lipotropin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 67 or SEQ ID NO: 68. In yet other aspects of this embodiment, a lipotropin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative

to SEQ ID NO: 67 or SEQ ID NO: 68. In yet other aspects of this embodiment, a lipotropin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 67 or SEQ ID NO: 68. In still other aspects of this embodiment, a lipotropin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 67 or SEQ ID NO: 68. In yet other aspects of this embodiment, a lipotropin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 67 or SEQ ID NO: 68.

[0268] In other aspects of this embodiment, a lipotropin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 67 or SEQ ID NO: 68. In other aspects of this embodiment, a lipotropin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 67 or SEQ ID NO: 68. In yet other aspects of this embodiment, a lipotropin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 67 or SEQ ID NO: 68. In yet other aspects of this embodiment, a lipotropin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 67 or SEQ ID NO: 68. In still other aspects of this embodiment, a lipotropin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 67 or SEQ ID NO: 68. In yet other aspects of this embodiment, a lipotropin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 67 or SEQ ID NO: 68.

[0269] In another embodiment, a melanocortin peptide comprising an altered targeting domain is a neuropeptide derived from a melanocortin peptide. In aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain is SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71.

[0270] In other aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71, at least 75% amino acid identity with SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71, at least 80% amino acid identity with SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71, at least 85% amino acid identity with SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71, at least 90% amino acid identity with SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71 or at least 95% amino acid identity with SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71. In yet other aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain has, *e.g.*, at most 70%

amino acid identity with SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71, at most 75% amino acid identity with SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71, at most 80% amino acid identity with SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71, at most 85% amino acid identity with SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71, at most 90% amino acid identity with SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71 or at most 95% amino acid identity with SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71.

[0271] In other aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71. In other aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71. In yet other aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71. In yet other aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71. In still other aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71. In yet other aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71.

[0272] In other aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71. In other aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71. In yet other aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71. In yet other aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71. In still other aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain has, *e.g.*, at least one,

two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71. In yet other aspects of this embodiment, a melanocortin peptide derived neuropeptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71.

[0273] In another embodiment, a peptide comprising an altered targeting domain is a galanin. In aspects of this embodiment, a galanin comprising an altered targeting domain is derived from a galanin or a galanin message-associated peptide (GMAP). In other aspects of this embodiment, a galanin comprising an altered targeting domain is SEQ ID NO: 72 or SEQ ID NO: 73.

[0274] In other aspects of this embodiment, a galanin comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 72 or SEQ ID NO: 73, at least 75% amino acid identity with SEQ ID NO: 72 or SEQ ID NO: 73, at least 80% amino acid identity with SEQ ID NO: 72 or SEQ ID NO: 73, at least 85% amino acid identity with SEQ ID NO: 72 or SEQ ID NO: 73, at least 90% amino acid identity with SEQ ID NO: 72 or SEQ ID NO: 73 or at least 95% amino acid identity with SEQ ID NO: 72 or SEQ ID NO: 73. In yet other aspects of this embodiment, a galanin comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 72 or SEQ ID NO: 73, at most 75% amino acid identity with SEQ ID NO: 72 or SEQ ID NO: 73, at most 80% amino acid identity with SEQ ID NO: 72 or SEQ ID NO: 73, at most 85% amino acid identity with SEQ ID NO: 72 or SEQ ID NO: 73, at most 90% amino acid identity with SEQ ID NO: 72 or SEQ ID NO: 73 or at most 95% amino acid identity with SEQ ID NO: 72 or SEQ ID NO: 73.

[0275] In other aspects of this embodiment, a galanin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 72 or SEQ ID NO: 73. In other aspects of this embodiment, a galanin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 72 or SEQ ID NO: 73. In yet other aspects of this embodiment, a galanin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 72 or SEQ ID NO: 73. In yet other aspects of this embodiment, a galanin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 72 or SEQ ID NO: 73. In still other aspects of this embodiment, a galanin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 72 or SEQ ID NO: 73. In yet other aspects of this embodiment, a galanin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 72 or SEQ ID NO: 73.

[0276] In other aspects of this embodiment, a galanin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 72 or SEQ ID NO: 73. In other aspects of this embodiment, a galanin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 72 or SEQ ID NO: 73. In yet other aspects of this embodiment, a galanin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 72 or SEQ ID NO: 73. In yet other aspects of this embodiment, a galanin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 72 or SEQ ID NO: 73. In still other aspects of this embodiment, a galanin comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 72 or SEQ ID NO: 73. In yet other aspects of this embodiment, a galanin comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 72 or SEQ ID NO: 73.

[0277] Another example of an altered targeting domain disclosed in the present specification is, *e.g.*, a granin peptide, such as, *e.g.*, a chromogranin A, a chromogranin B (secretogranin I) or a chromogranin C (secretogranin II). Thus, in an embodiment, an altered targeting domain is derived from a granin peptide.

[0278] In another embodiment, a granin peptide comprising an altered targeting domain is a chromogranin A peptide. In aspects of this embodiment, a chromogranin A peptide comprising an altered targeting domain is derived from a β-granin, a vasostatin, a chromostatin, a pancreastatin, a WE-14, a catestatin, a parastatin or a GE-25. In other aspects of this embodiment, a chromogranin A peptide comprising an altered targeting domain is SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81.

[0279] In other aspects of this embodiment, a chromogranin A peptide comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81, at least 75% amino acid identity with SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81, at least 80% amino acid identity with SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81, at least 85% amino acid identity with SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81, at least 90% amino acid identity with SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81 or at least 95% amino acid identity with SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81. In yet other aspects of this embodiment, a chromogranin A peptide comprising an altered

targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81, at most 75% amino acid identity with SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81, at most 80% amino acid identity with SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81, at most 85% amino acid identity with SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81, at most 90% amino acid identity with SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81 or at most 95% amino acid identity with SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81.

[0280] In other aspects of this embodiment, a chromogranin A peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81. In other aspects of this embodiment, a chromogranin A peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81. In yet other aspects of this embodiment, a chromogranin A peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81. In yet other aspects of this embodiment, a chromogranin A peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81. In still other aspects of this embodiment, a chromogranin A peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81. In yet other aspects of this embodiment, a chromogranin A peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81.

[0281] In other aspects of this embodiment, a chromogranin A peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81. In other aspects of this embodiment, a

chromogranin A peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81. In yet other aspects of this embodiment, a chromogranin A peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81. In yet other aspects of this embodiment, a chromogranin A peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81. In still other aspects of this embodiment, a chromogranin A peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81. In yet other aspects of this embodiment, a chromogranin A peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81.

[0282] In another embodiment, a granin peptide comprising an altered targeting domain is a chromogranin B peptide. In aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain is derived from a GAWK peptide, an adrenomedullary peptide or a secretolytin. In other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain is SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86.

[0283] In other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86, at least 75% amino acid identity with SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86, at least 80% amino acid identity with SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86, at least 85% amino acid identity with SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86, at least 90% amino acid identity with SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86 or at least 95% amino acid identity with SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86. In yet other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86, at most 75% amino acid identity with SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86, at most 80% amino acid identity with SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86, at most 85% amino acid identity with SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86, at

most 90% amino acid identity with SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86 or at most 95% amino acid identity with SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86.

[0284] In other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86. In other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86. In yet other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86. In yet other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86. In still other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86. In yet other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86.

[0285] In other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86. In other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86. In yet other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86. In yet other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86. In still other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86.

In yet other aspects of this embodiment, a chromogranin B peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 or SEQ ID NO: 86.

[0286] In another embodiment, a granin peptide comprising an altered targeting domain is a chromogranin C peptide. In aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain is derived from a secretoneurin. In other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain is SEQ ID NO: 87.

[0287] In other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 87, at least 75% amino acid identity with SEQ ID NO: 87, at least 80% amino acid identity with SEQ ID NO: 87, at least 85% amino acid identity with SEQ ID NO: 87, at least 90% amino acid identity with SEQ ID NO: 87 or at least 95% amino acid identity with SEQ ID NO: 87. In yet other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 87, at most 75% amino acid identity with SEQ ID NO: 87, at most 80% amino acid identity with SEQ ID NO: 87, at most 85% amino acid identity with SEQ ID NO: 87, at most 90% amino acid identity with SEQ ID NO: 87 or at most 95% amino acid identity with SEQ ID NO: 87.

[0288] In other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 87. In other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 87. In yet other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 87. In yet other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 87. In still other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 87. In yet other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 87.

[0289] In other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 87. In other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine

or ten contiguous amino acid substitutions relative to SEQ ID NO: 87. In yet other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 87. In yet other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 87. In still other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 87. In yet other aspects of this embodiment, a chromogranin C peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 87.

[0290] Another example of an altered targeting domain disclosed in the present specification is, *e.g.*, a tachykinin peptide, such as, *e.g.*, a Substance P, a neuropeptide K (NPK), a neuropeptide gamma (NP gamma), a neurokinin A (NKA; Substance K, neurokinin alpha, neuromedin L), a neurokinin B (NKB), a hemokinin or a endokinin. Thus, in an embodiment, an altered targeting domain is derived from a tachykinin peptide.

[0291] In aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain is derived from a Substance P, a neuropeptide K (NPK), a neuropeptide gamma (NP gamma), a neurokinin A (NKA; Substance K, neurokinin alpha, neuromedin L), a neurokinin B (NKB), a hemokinin or a endokinin. In other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain is SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 or SEQ ID NO: 99.

[0292] In other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99, at least 75% amino acid identity with SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99, at least 80% amino acid identity with SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99, at least 85% amino acid identity with SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99, at least 90% amino acid identity with SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99 or at least 95% amino acid identity with

SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99. In yet other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99, at most 75% amino acid identity with SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99, at most 80% amino acid identity with SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99, at most 85% amino acid identity with SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99, at most 90% amino acid identity with SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99 or at most 95% amino acid identity with SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99.

[0293] In other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid substitutions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99. In other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid substitutions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99. In yet other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid deletions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99. In yet other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid deletions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99. In still other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid additions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99. In yet other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid

additions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99.

[0294] In other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid substitutions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99. In other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid substitutions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99. In yet other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid deletions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99. In yet other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid deletions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99. In still other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid additions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99. In yet other aspects of this embodiment, a tachykinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid additions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 OR SEQ ID NO: 99.

[0295] Another example of an altered targeting domain disclosed in the present specification is, *e.g.*, a cholecystokinin peptide, such as, *e.g.*, a cholecystokinin 58, a cholecystokinin 39, a cholecystokinin 33, a cholecystokinin 12 or a cholecystokinin 8. Thus, in an embodiment, an altered targeting domain is derived from a cholecystokinin peptide.

[0296] In aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain is derived from a cholecystokinin 58, a cholecystokinin 39, a cholecystokinin 33, a cholecystokinin 12 or a cholecystokinin 8. In other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain is SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115. In

still other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain comprises amino acids 20-58 of SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain comprises amino acids 26-58 of SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115. In still further other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain comprises amino acids 47-58 of SEQ ID NO: 100, SEQ ID NO: 110 or SEQ ID NO: 114. In yet further aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain comprises amino acids 51-58 of SEQ ID NO: 100.

[0297] In other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115, at least 75% amino acid identity with SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115, at least 80% amino acid identity with SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115, at least 85% amino acid identity with SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115, at least 90% amino acid identity with SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115 or at least 95% amino acid identity with SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115, at most 75% amino acid identity with SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ

ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115, at most 80% amino acid identity with SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115, at most 85% amino acid identity with SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115, at most 90% amino acid identity with SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115 or at most 95% amino acid identity with SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115.

[0298] In other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115. In other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115. In still other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO:

104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115.

[0299] In other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115. In other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115. In still other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115.

[0300] In still other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100, at least 75% amino acid identity with amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100, at least 80% amino acid identity with amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100, at least 85% amino acid identity with amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100, at least 90% amino acid identity with amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100 or at least 95% amino acid identity with amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100, at most 75% amino acid identity with amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100, at most 80% amino acid identity with amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100, at most 85% amino acid identity with amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100, at most 90% amino acid identity with amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100 or at most 95% amino acid identity with amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100.

[0301] In still other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100. In other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100. In still other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100.

[0302] In still other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100. In other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100. In still other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to amino acids 20-58 of SEQ ID NO: 100 or amino acids 26-58 of SEQ ID NO: 100.

[0303] In other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100, at least 75% amino acid identity with amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100, at least 80% amino acid identity with amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100, at least 85% amino acid identity with amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100, at least 90% amino acid identity with amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100 or at least 95% amino acid identity with amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100, at most 75% amino acid identity with amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100, at most 80% amino acid identity with amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100, at most 85% amino acid identity with amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100, at most 90% amino acid identity with amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100 or at most 95% amino acid identity with amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100.

[0304] In other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100. In other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three or four non-contiguous amino acid deletions relative to amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three or four non-contiguous amino acid deletions relative to amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100. In still other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three or four non-contiguous amino acid additions relative to amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three or four non-contiguous amino acid additions relative to amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100.

[0305] In other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three or four contiguous amino acid substitutions relative to amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100. In other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three or four contiguous amino acid substitutions relative to amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three or four contiguous amino acid deletions relative to amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three or four contiguous amino acid deletions relative to amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100. In still other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three or four contiguous amino acid additions relative to amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100. In yet other aspects of this embodiment, a cholecystokinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three or four contiguous amino acid additions relative to amino acids 47-58 of SEQ ID NO: 100 or amino acids 51-58 of SEQ ID NO: 100.

[0306] Another example of an altered targeting domain disclosed in the present specification is, *e.g.*, a Neuropeptide Y related peptide, such as, *e.g.*, a Neuropeptide Y (NPY), a Peptide YY (PYY), Pancreatic

peptide (PP) or a Pancreatic icosapeptide (PIP). Thus, in an embodiment, an altered targeting domain is derived from a Neuropeptide Y related peptide.

[0307] In aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain is derived from a Neuropeptide Y (NPY), a Peptide YY (PYY), Pancreatic peptide (PP) or a Pancreatic icosapeptide (PIP). In other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain is SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120.

[0308] In other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120, at least 75% amino acid identity with SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120, at least 80% amino acid identity with SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120, at least 85% amino acid identity with SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120, at least 90% amino acid identity with SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120 or at least 95% amino acid identity with SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120. In yet other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120, at most 75% amino acid identity with SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120, at most 80% amino acid identity with SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120, at most 85% amino acid identity with SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120, at most 90% amino acid identity with SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120 or at most 95% amino acid identity with SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120.

[0309] In other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120. In other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120. In yet other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120. In yet other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three,

four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120. In still other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120. In yet other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120.

[0310] In other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120. In other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120. In yet other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120. In yet other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120. In still other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120. In yet other aspects of this embodiment, a Neuropeptide Y related peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120.

[0311] Another example of an altered targeting domain disclosed in the present specification is, *e.g.*, a corticotropin-releasing hormone, a thyrotropin-releasing hormone, somatostatin, a leukemia inhibitor factor (LIF) or an interlukin-1 (IL1). Thus, in an embodiment, an altered targeting domain is derived from a corticotropin-releasing hormone. In another embodiment, an altered targeting domain is derived from a thyrotropin-releasing hormone. In another embodiment, an altered targeting domain is derived from a somatostatin. In another embodiment, an altered targeting domain is derived from a LIF. In another embodiment, an altered targeting domain is derived from an IL1. In aspects of this embodiment, a peptide comprising an altered targeting domain is SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187.

[0312] In other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 178, SEQ ID NO: 183 or SEQ ID NO: 184, at least 75% amino acid identity with SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187, at least 80% amino acid identity with SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187, at least 85% amino acid identity with SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187, at least 90% amino acid identity with SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187 or at least 95% amino acid identity with SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187. In yet other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187, at most 75% amino acid identity with SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187, at most 80% amino acid identity with SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187, at most 85% amino acid identity with SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187, at most 90% amino acid identity with SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187 or at most 95% amino acid identity with SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187.

[0313] In other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid substitutions relative to SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187. In other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid substitutions relative to SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187. In yet other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid deletions relative to SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187. In yet other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid deletions relative to SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187. In still other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid additions relative to SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187. In yet other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid

additions relative to SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187.

[0314] In other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid substitutions relative to SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187. In other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid substitutions relative to SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187. In yet other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid deletions relative to SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187. In yet other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid deletions relative to SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187. In still other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid additions relative to SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187. In yet other aspects of this embodiment, a peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid additions relative to SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 or SEQ ID NO: 187.

[0315] Another example of an altered targeting domain disclosed in the present specification is a kinin peptide, such as, *e.g.*, a bradykinin, a kallidin, a desArg^{9} bradykinin and a desArg^{10} bradykinin. Thus, in an embodiment, an altered targeting domain is derived from a kinin peptide. In aspects of this embodiment, a kinin peptide comprising an altered targeting domain is derived from a bradykinin, a kallidin, a desArg^{9} bradykinin and a desArg^{10} bradykinin. In other aspects of this embodiment, a kinin peptide comprising an altered targeting domain comprises SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181.

[0316] In other aspects of this embodiment, a kinin peptide comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181, at least 75% amino acid identity with SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181, at least 80% amino acid identity with SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181, at least 85% amino acid identity with SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181, at least 90% amino acid identity with SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181 or at least 95% amino acid identity with SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181. In yet other aspects of this embodiment, a kinin peptide comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181, at most 75% amino acid identity with SEQ ID NO:

178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181, at most 80% amino acid identity with SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181, at most 85% amino acid identity with SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181, at most 90% amino acid identity with SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181 or at most 95% amino acid identity with SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181.

[0317] In other aspects of this embodiment, a kinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181. In other aspects of this embodiment, a kinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid substitutions relative to SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181. In yet other aspects of this embodiment, a kinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181. In yet other aspects of this embodiment, a kinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid deletions relative to SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181. In still other aspects of this embodiment, a kinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181. In yet other aspects of this embodiment, a kinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181.

[0318] In other aspects of this embodiment, a kinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181. In other aspects of this embodiment, a kinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid substitutions relative to SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181. In yet other aspects of this embodiment, a kinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181. In yet other aspects of this embodiment, a kinin peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid deletions relative to SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181. In still other aspects of this embodiment, a kinin peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181. In yet other aspects of this embodiment, a kinin peptide comprising an altered targeting domain has, *e.g.*, at

most one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 or SEQ ID NO: 181.

[0319] Another example of an altered targeting domain disclosed in the present specification is a PAR peptide, such as, *e.g.*, a PAR1 peptide, a PAR2 peptide, a PAR3 peptide and a PAR4 peptide. Thus, in an embodiment, an altered targeting domain is derived from a PAR peptide. In aspects of this embodiment, a PAR peptide comprising an altered targeting domain is derived from a PAR1 peptide, a PAR2 peptide, a PAR3 peptide or a PAR4 peptide. In other aspects of this embodiment, a PAR peptide comprising an altered targeting domain comprises amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

[0320] In other aspects of this embodiment, a PAR peptide comprising an altered targeting domain has, *e.g.*, at least 70% amino acid identity with amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185, at least 75% amino acid identity with amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185, at least 80% amino acid identity with amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185, at least 85% amino acid identity with amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185, at least 90% amino acid identity with amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185 or at least 95% amino acid identity with amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60

of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

[0321] In yet other aspects of this embodiment, a PAR peptide comprising an altered targeting domain has, *e.g.*, at most 70% amino acid identity with amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185, at most 75% amino acid identity with amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185, at most 80% amino acid identity with amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185, at most 85% amino acid identity with amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185, at most 90% amino acid identity with amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185 or at most 95% amino acid identity with amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

[0322] In other aspects of this embodiment, a PAR peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid substitutions relative to amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185. In other aspects of this embodiment, a PAR peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid substitutions relative to amino acids 42-47, amino acids 42-55,

amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185. In yet other aspects of this embodiment, a PAR peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid deletions relative to amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185. In yet other aspects of this embodiment, a PAR peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid deletions relative to amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185. In still other aspects of this embodiment, a PAR peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five non-contiguous amino acid additions relative to amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185. In yet other aspects of this embodiment, a PAR peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five non-contiguous amino acid additions relative to amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

[0323] In other aspects of this embodiment, a PAR peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid substitutions relative to amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185. In other aspects of this embodiment, a PAR peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid substitutions relative to amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids

1-70 of SEQ ID NO: 185. In yet other aspects of this embodiment, a PAR peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid deletions relative to amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185. In yet other aspects of this embodiment, a PAR peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid deletions relative to amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185. In still other aspects of this embodiment, a PAR peptide comprising an altered targeting domain has, *e.g.*, at least one, two, three, four or five contiguous amino acid additions relative to amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185. In yet other aspects of this embodiment, a PAR peptide comprising an altered targeting domain has, *e.g.*, at most one, two, three, four or five contiguous amino acid additions relative to amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

[0324] An altered targeting domain disclosed in the present specification replaces the binding activity of the Clostridial toxin targeting domain found in naturally occurring Clostridial toxins. As used herein, the term "Clostridial toxin targeting domain" is synonymous with "Clostridial toxin H_{CC} targeting region" or "Clostridial toxin H_{CC} region" and means any naturally occurring Clostridial toxin polypeptide that can execute the cell binding step of the intoxication process, including, *e.g.*, the binding of the Clostridial toxin to a Clostridial toxin-specific receptor located on the plasma membrane surface of a target cell. It is envisioned that replacement of the binding activity can be achieved by, *e.g.*, replacing the entire Clostridial toxin H_{CC} targeting domain with an altered targeting domain; replacing a portion of a Clostridial toxin H_{CC} targeting domain with an altered targeting domain, with the proviso that the portion of a Clostridial toxin H_{CC} targeting domain remaining cannot selectively bind to its Clostridial toxin receptor; and operably-linking an altered targeting domain to a Clostridial toxin comprising a Clostridial toxin H_{CC} targeting domain, with the proviso that the a Clostridial toxin H_{CC} targeting domain is altered so that it cannot selectively bind to its Clostridial toxin receptor.

[0325] The three-dimensional crystal structures of BoNT/A, BoNT/B and the H_C domain of TeNT indicate

that the three functional domains of Clostridial neurotoxins are structurally distinct. The HEXXH consensus motif of the light chain forms the tetrahedral zinc binding pocket of the catalytic site located in a deep cleft on the protein surface that is accessible by a channel. The structure of the H_N and H_C domains consists primarily of β-sheet topologies that are linked by a single α-helix. The cylindrical-shaped H_N domain comprises two long amphipathic α-helices that resemble the coiled-coil motif found in some viral proteins. The H_N domain also forms a long unstructured loop called the 'translocation belt,' which wraps around a large negatively charged cleft of the light chain that blocks access of the zinc atom to the catalytic-binding pocket of active site. The H_C domain comprises two distinct structural features of roughly equal size that indicate function. The first, designated the H_{CN} domain, is located in the amino half of the H_C domain. The H_{CN} domain forms a β-barrel, jelly-roll fold. The H_{CC} domain is the second domain that comprises the H_C domain. This carboxyl-terminal domain comprises a modified β-trefoil domain which forms three distinct carbohydrate binding regions that resembles the carbohydrate binding moiety found in many sugar-binding proteins, such as, *e.g.*, serum amyloid P, sialidase, cryia, insecticidal ∂-endotoxin and lectins. Biochemical studies indicate that the β-trefoil domain structure of the H_{CC} domain appears to mediate the binding to specific carbohydrate containing components of the Clostridial toxin receptor on the cell surface, see, *e.g.*, Krzysztof Ginalski et al., *Structure-based Sequence Alignment for the Beta-Trefoil Subdomain of the Clostridial Neurotoxin Family Provides Residue Level Information About the Putative Ganglioside Binding Site*, 482(1-2) FEBS Lett. 119-124 (2000). The H_C domain tilts away from the H_N domain exposing the surface loops and making them accessible for binding. No contacts occur between the light chain and the H_C domain.

[0326] Proteins containing the structural β-trefoil domain represents a diverse group of proteins, see, *e.g.*, C. A. Orengo et al., *Protein Superfamilies and Domain Superfolds*, 372 Nature 631-634 (1994). The β-trefoil domain comprises a six-stranded β-barrel closed off at one end by three β-hairpin structures that exhibits a characteristic pseudo-threefold axis symmetry. The monomeric structural unit of this three-fold symmetry is referred to as the β-trefoil fold that contains four β-sheets organized as a pair of antiparallel β-sheets. Dividing each of these β-trefoil folds is a β-hairpin turn. Therefore, in a linear fashion, a β-trefoil domain comprises four β-sheets of the first β-trefoil fold, a β-hairpin turn, four β-sheets of the second β-trefoil fold, a second β-hairpin turn four β-sheets of the third β-trefoil fold. Because the first hairpin turn is located between the fourth and fifth β-sheets of the β-trefoil domain, it is designated the β4/β5 β-hairpin turn. Likewise, since the second hairpin turn is located between the eight and ninth β-sheets of the β-trefoil domain, it is designated the β8/β9 β-hairpin turn.

[0327] As is typical for proteins containing a β-trefoil fold, the overall amino acid sequence identity of the H_{CC} domain between Clostridial toxins is low. However, key residues essential for binding activity have been identified by structural analysis and mutagenesis experiments, see, *e.g.*, Krzysztof Ginalski et al., *Structure-based Sequence Alignment for the Beta-Trefoil Subdomain of the Clostridial Neurotoxin Family Provides Residue Level Information About the Putative Ganglioside Binding Site*, 482(1-2) FEBS Lett. 119-124 (2000); and Andreas Rummel et al., *The H_{CC}-Domain of Botulinum Neurotoxins A and B Exhibits*

a Singular Ganglioside Binding Site Displaying Serotype Specific Carbohydrate Interaction, 51(3) Mol. Microbiol. 631-643 (2004). Additionally, research has elucidated that β4/β5 and β8/β9 β-hairpin turns are important in conferring the proper pseudo-threefold axis symmetry observed in the β-trefoil domain and that these turns are important for β-trefoil domain stability, see, *e.g.*, Stephen R. Brych et al., *Structure and Stability Effects of Mutations Designed to Increase the Primary Sequence Symmetry Within the Core Region of a β-trefoil*, 10 Protein Sci. 2587-2599 (2001); Jaewon Kim et al., *Alternative Type I and I' Turn Conformations in the β8/β9 β-hairpin of Human Acidic Fibroblast Growth Factor*, 11 Protein Sci. 459-466 (2002); Jaewon Kim et al., *Sequence swapping Does Not Result in Conformation Swapping for the β4/β5 and β8/β9 β-hairpin Turns in Human Acidic Fibroblast Growth Factor*, 14 Protein Sci. 351-359 (2005). The amino acid sequences comprising the ß-trefoil domains found in various Clostridial toxins are shown in Table 2.

Table 2. β-trefoil Domains of Clostridial Toxins						
Protein	SEQ ID NO:	Amino Acid Sequence Region of Carbohydrate Binding Moieties				
		α-fold	β4/β5 β-hairpin turn	ß-fold	β8/β9 β-hairpin turn	γ-fold
BoNT/A	1	1111-1162	1163-1178	1179-1223	1224-1236	1237-1296
BoNT/B	2	1098-1147	1148-1165	1166-1210	1211-1222	1223-1291
BoNT/C1	3	1112-1150	1151-1166	1167-1218	1219-1229	1230-1291
BoNT/D	4	1099-1137	1138-1153	1154-1207	1208-1218	1219-1276
BoNT/E	5	1086-1129	1130-1146	1147-1190	1191-1198	1199-1252
BoNT/F	6	1106-1152	1153-1171	1172-1213	1214-1221	1222-1274
BoNT/G	7	1106-1153	1154-1172	1173-1218	1219-1230	1231-1297
TeNT	8	1128-1177	1178-1194	1195-1240	1241-1254	1255-1315

[0328] Thus, in an embodiment, a Clostridial toxin targeting domain comprising an H_{CC} region can be replaced with an enhance binding domain disclosed in the present specification. In aspects of this embodiment, a BoNT/A H_{CC} region can be replaced with an altered targeting domain, a BoNT/B H_{CC} region can be replaced with an altered targeting domain, a BoNT/C1 H_{CC} region can be replaced with an altered targeting domain, a BoNT/D H_{CC} region can be replaced with an altered targeting domain, a BoNT/E H_{CC} region can be replaced with an altered targeting domain, a BoNT/F H_{CC} region can be replaced with an altered targeting domain, a BoNT/G H_{CC} region can be replaced with an altered targeting domain and a TeNT H_{CC} region can be replaced with an altered targeting domain.

[0329] In aspects of this embodiment, a BoNT/A H_{CC} region can be replaced with an altered targeting domain, a BoNT/B H_{CC} region can be replaced with an altered targeting domain, a BoNT/C1 H_{CC} region can be replaced with an altered targeting domain, a BoNT/D H_{CC} region can be replaced with an altered targeting domain, a BoNT/E H_{CC} region can be replaced with an altered targeting domain, a BoNT/F H_{CC} region can be replaced with an altered targeting domain, a BoNT/G H_{CC} region can be replaced with an altered targeting domain and a TeNT H_{CC} region can be replaced with an altered targeting domain. In other aspects of this embodiment, a BoNT/A H_{CC} region comprising amino acids 1092-1296 of SEQ ID NO: 1 can be replaced with an altered targeting domain, a BoNT/B H_{CC} region comprising amino acids 1079-1291 of SEQ ID NO: 2 can be replaced with an altered targeting domain, a BoNT/C1 H_{CC} region comprising amino acids 1093-1291 of SEQ ID NO: 3 can be replaced with an altered targeting domain, a BoNT/D H_{CC} region comprising amino acids 1080-1276 of SEQ ID NO: 4 can be replaced with an altered targeting domain, a BoNT/E H_{CC} region comprising amino acids 1067-1252 of SEQ ID NO: 5 can be replaced with an altered targeting domain, a BoNT/F H_{CC} region comprising amino acids 1087-1274 of SEQ ID NO: 6 can be replaced with an altered targeting domain, a BoNT/G H_{CC} region comprising amino acids 1087-1297 of SEQ ID NO: 7 can be replaced with an altered targeting domain and a TeNT H_{CC} region comprising amino acids 1109-1315 of SEQ ID NO: 8 can be replaced with an altered targeting domain.

[0330] In another embodiment, an altered binding domain disclosed in the present specification is operably-linked to a Clostridial toxin comprising a Clostridial toxin targeting domain altered so that it cannot selectively bind to its Clostridial toxin receptor. As used herein, the term "altered," when referring to a Clostridial toxin targeting domain, means a naturally occurring Clostridial toxin targeting domain modified to eliminate or reduce the binding activity of the Clostridial toxin targeting domain so that the domain can no longer selectively bind to its Clostridial toxin receptor. By definition, an altered Clostridial toxin targeting domain has at least one amino acid change from the corresponding region of the disclosed reference sequences (see Table 1) and can be described in percent identity to the corresponding region of that reference sequence. As non-limiting examples, a modified BoNT/A H_{CC} region comprising amino acids 1111-1296 of SEQ ID NO: 1 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1111-1296 of SEQ ID NO: 1; a modified BoNT/B H_{CC} region comprising amino acids 1098-1291 of SEQ ID NO: 2 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1098-1291 of SEQ ID NO: 2; a modified BoNT/C1 H_{CC} region comprising amino acids 1112-1291 of SEQ ID NO: 3 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1112-1291 of SEQ ID NO: 3; a modified BoNT/D H_{CC} region comprising amino acids 1099-1276 of SEQ ID NO: 4 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1099-1276 of SEQ ID NO: 4; a modified BoNT/E H_{CC} region comprising amino acids 1086-1252 of SEQ ID NO: 5 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1086-1252 of SEQ ID NO: 5; a modified BoNT/F H_{CC} region comprising amino acids 1106-1274 of SEQ ID NO: 6 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1106-1274 of SEQ ID NO: 6; a modified BoNT/G H_{CC} region comprising amino acids 1106-1297 of SEQ ID NO: 7 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1106-1297 of SEQ ID NO: 7; and a modified TeNT H_{CC} region comprising amino acids 1128-1315 of SEQ ID NO: 8 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1128-1315 of SEQ ID NO: 8.

[0331] In aspects of this embodiment, an altered Clostridial toxin H_{CC} targeting region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with its reference sequence, at least 75% amino acid identity with its reference sequence, at least 80% amino acid identity with its reference sequence, at least 85% amino acid identity with its reference sequence, at least 90% amino acid identity with its reference sequence or at least 95% amino acid identity with its reference sequence. In yet other aspects of this embodiment, an altered Clostridial toxin H_{CC} targeting region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with its reference sequence, at most 75% amino acid identity with its reference sequence, at most 80% amino acid identity with its reference sequence, at most 85% amino

acid identity with its reference sequence, at most 90% amino acid identity with its reference sequence or at most 95% amino acid identity with its reference sequence.

[0332] In other aspects of this embodiment, an altered Clostridial toxin H_{CC} targeting region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, or 200 non-contiguous amino acid substitutions relative to its reference sequence. In other aspects of this embodiment, an altered Clostridial toxin H_{CC} targeting region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid substitutions relative to its reference sequence. In yet other aspects of this embodiment, an altered Clostridial toxin H_{CC} targeting region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to its reference sequence. In other aspects of this embodiment, an altered Clostridial toxin H_{CC} targeting region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid deletions relative to its reference sequence. In still other aspects of this embodiment, an altered Clostridial toxin H_{CC} targeting region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to its reference sequence. In other aspects of this embodiment, an altered Clostridial toxin H_{CC} targeting region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 non-contiguous amino acid additions relative to its reference sequence.

[0333] In other aspects of this embodiment, an altered Clostridial toxin H_{CC} targeting region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to its reference sequence. In other aspects of this embodiment, an altered Clostridial toxin H_{CC} targeting region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid substitutions relative to its reference sequence. In yet other aspects of this embodiment, an altered Clostridial toxin H_{CC} targeting region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to its reference sequence. In other aspects of this embodiment, an altered Clostridial toxin H_{CC} targeting region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid deletions relative to its reference sequence. In still other aspects of this embodiment, an altered Clostridial toxin H_{CC} targeting region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to its reference sequence. In other aspects of this embodiment, an altered Clostridial toxin H_{CC} targeting region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100 or 200 contiguous amino acid additions relative to its reference sequence.

[0334] In another aspect of the invention, a modified Clostridial toxin with an altered targeting activity comprises, in part, a protease cleavage site is located within a di-chain loop region. As used herein, the term "di-chain loop region" means the amino acid sequence of a Clostridial toxin containing a protease cleavage site used to convert the single-chain form of a Clostridial toxin into the di-chain form. Non-limiting examples of a Clostridial toxin di-chain loop region, include, a di-chain loop region of BoNT/A comprising amino acids 430-454 of SEQ ID NO: 1; a di-chain loop region of BoNT/B comprising amino acids 437-446 of SEQ ID NO: 2; a di-chain loop region of BoNT/C1 comprising amino acids 437-453 of SEQ ID NO: 3; a di-chain loop region of BoNT/D comprising amino acids 437-450 of SEQ ID NO: 4; a di-chain loop region of BoNT/E comprising amino acids 412-426 of SEQ ID NO: 5; a di-chain loop region of BoNT/F comprising amino acids 429-445 of SEQ ID NO: 6; a di-chain loop region of BoNT/G comprising amino acids 436-450 of SEQ ID NO: 7; and a di-chain loop region of TeNT comprising amino acids 439-467 of SEQ ID NO: 8 (Table 3).

Table 3. Di-chain Loop Region of Clostridial Toxins

Toxin	SEQ ID NO:	Light Chain Region	Di-chain Loop Region Containing the Naturally-occurring Protease Cleavage Site	Heavy Chain Region
BoNT/A	1	NMNFTKLKNFTGLFEFYKLL	CVRGIITSKTKSLDKGYNK*---ALNDLC	IKVNNWDL
BoNT/B	2	KQAYEEISKEHLAVYKIQM	CKSVK*----------APGIC	IDVDNEDL
BoNT/C1	3	PALRKVNPENMLYLFTKF	CHKAIDGRSLYNK*---------TLDC	RELLVKNTDL
BoNT/D	4	PALQKLSSESVVDLFTKV	CLRLTKNSR*----------DDSTC	IKVKNNRL
BoNT/E	5	IITPITGRGLVKKIIRF	CKNIVSVKGIR*----------KSIC	IEINNGEL
BoNT/F	6	IIDSIPDKGLVEKIVKF	CKSVIPRKGTK*---------APPRLC	IRVNNSEL
BoNT/G	7	KEAYEEISLEHLVIYRIAM	CKPVMYKNTGK*----------SEQC	IIVNNEDL
TeNT	8	TNAFRNVDGSGLVSKLIGL	CKKIIPPTNIRENLYNRTA*SLTDLGGELC	IKIKNEDL

The amino acid sequence displayed are as follows: BoNT/A, residues 325-462 of SEQ ID No: 1; BoNT/B, residues 332-454 of SEQ ID No: 2; BoNT/C1, residues 334-463 of SEQ ID No: 3; BoNT/D, residues 334-458 of SEQ ID No: 4; BoNT/E, residues 311-434 of SEQ ID No: 5; BoNT/F, residues 328-453 of SEQ ID No: 6; BoNT/G, residues 331-458 of SEQ ID No: 7; and TeNT, residues 334-474 of SEQ ID No: 8. An asterisks (*) indicates the peptide bond that is cleaved by a Clostridial toxin protease.

[0335] In is envisioned that any and all protease cleavage sites can be used to convert the single-chain polypeptide form of a Clostridial toxin into the di-chain form, including, without limitation, endogenous di-chain loop protease cleavage sites and exogenous protease cleavage sites. The location and kind of protease cleavage site may be critical because certain targeting domains require a free amino-terminal or carboxyl-terminal amino acid. For example, when a targeting domain is placed between two other domains, *e.g.*, see FIG. 5, a criteria for selection of a protease cleavage site could be whether the protease that cleaves its site leaves a flush cut, exposing the free amino-terminal or carboxyl-terminal of the altered targeting domain necessary for selective binding of the targeting domain to its receptor. The selection and placement of a protease cleavage site is well known in the art.

[0336] As used herein, the term "endogenous di-chain loop protease cleavage site" is synonymous with a "naturally occurring di-chain loop protease cleavage site" and means a naturally occurring protease cleavage site found within the di-chain loop region of a naturally occurring Clostridial toxin and includes, without limitation, naturally occurring Clostridial toxin di-chain loop protease cleavage site variants, such as, *e.g.*, Clostridial toxin di-chain loop protease cleavage site isoforms and Clostridial toxin di-chain loop protease cleavage site subtypes. Non-limiting examples of an endogenous protease cleavage site, include, *e.g.*, a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site and a TeNT di-chain loop protease cleavage site.

[0337] As mentioned above, Clostridial toxins are translated as a single-chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulfide loop by a naturally-occurring protease. This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by a single disulphide bond and noncovalent interactions. While the identity of the protease is currently unknown, the di-chain loop protease cleavage site for many Clostridial toxins has been proposed. In BoNTs, cleavage at K448-A449 converts the single polypeptide form of BoNT/A into the di-chain form; cleavage at K441-A442 converts the single polypeptide form of BoNT/B into the di-chain form; cleavage at K449-T450 converts the single polypeptide form of BoNT/C1 into the di-chain form; cleavage at R445-D446 converts the single polypeptide form of BoNT/D into the di-chain form; cleavage at R422-K423 converts the single polypeptide form of BoNT/E into the di-chain form; cleavage at K439-A440 converts the single polypeptide form of BoNT/F into the di-chain form; and cleavage at K446-S447 converts the single polypeptide form of BoNT/G into the di-chain form. Proteolytic cleavage of the single polypeptide form of TeNT at A457-S458 results in the di-chain form. Such a di-chain loop protease cleavage site is operably-linked in-frame to a modified Clostridial toxin as a fusion protein.

[0338] However, it should also be noted that additional cleavage sites within the di-chain loop also appear to be cleaved resulting in the generation of a small peptide fragment being lost. As a non-limiting example, BoNT/A single-chain polypeptide cleave ultimately results in the loss of a ten amino acid fragment within the di-chain loop. Thus, in BoNTs, cleavage at S441-L442 converts the single polypeptide form of BoNT/A into the di-chain form; cleavage at G444-I445 converts the single polypeptide form of BoNT/B into the di-chain form; cleavage at S445-L446 converts the single polypeptide form of BoNT/C1 into the di-chain form; cleavage at K442-N443 converts the single polypeptide form of BoNT/D into the di-chain form; cleavage at K419-G420 converts the single polypeptide form of BoNT/E into the di-chain form; cleavage at K423-S424 converts the single polypeptide form of BoNT/E into the di-chain form; cleavage at K436-G437 converts the single polypeptide form of BoNT/F into the di-chain form; cleavage at T444-G445 converts the single polypeptide form of BoNT/G into the di-chain form; and cleavage at E448-Q449 converts the single polypeptide form of BoNT/G into the di-chain form.

[0339] Thus, in an embodiment, a protease cleavage site comprising an endogenous Clostridial toxin di-chain loop protease cleavage site is used to convert the single-chain toxin into the di-chain form. In aspects of this embodiment, conversion into the di-chain form by proteolytic cleavage occurs from a site comprising, *e.g.*, a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site or a TeNT di-chain loop protease cleavage site.

[0340] In other aspects of this embodiment, conversion into the di-chain form by proteolytic cleavage occurs from a site comprising, *e.g.*, a di-chain loop region of BoNT/A comprising amino acids 430-454 of SEQ ID NO: 1; a di-chain loop region of BoNT/B comprising amino acids 437-446 of SEQ ID NO: 2; a di-chain loop region of BoNT/C1 comprising amino acids 437-453 of SEQ ID NO: 3; a di-chain loop region of BoNT/D comprising amino acids 437-450 of SEQ ID NO: 4; a di-chain loop region of BoNT/E comprising amino acids 412-426 of SEQ ID NO: 5; a di-chain loop region of BoNT/F comprising amino acids 429-445 of SEQ ID NO: 6; a di-chain loop region of BoNT/G comprising amino acids 436-450 of SEQ ID NO: 7; or a di-chain loop region of TeNT comprising amino acids 439-467 of SEQ ID NO: 8.

[0341] It is also envisioned that an exogenous protease cleavage site can be used to convert the single-chain polypeptide form of a modified Clostridial toxin disclosed in the present specification into the di-chain form. As used herein, the term "exogenous protease cleavage site" is synonymous with a "non-naturally occurring protease cleavage site" and means a protease cleavage site that is not normally present in a di-chain loop region from a naturally occurring Clostridial toxin. Non-limiting examples of exogenous protease cleavage sites include, *e.g.*, an enterokinase cleavage site (Table 4); a Thrombin cleavage site (Table 4); a Factor Xa cleavage site (Table 4); a human rhinovirus 3C protease cleavage site (Table 4); a tobacco etch virus (TEV) protease cleavage site (Table 4); a dipeptidyl aminopeptidase cleavage site; a small ubiquitin-like modifier (SUMO)/ubiquitin-like protein-1(ULP-1) protease cleavage site, such as, *e.g.*, MADSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGK EMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGG (SEQ ID. NO: 142); and a Clostridial toxin substrate cleavage site.

[0342] As mentioned above, a Clostridial toxin is converted from a single polypeptide form into a di-chain molecule by proteolytic cleavage. While the naturally-occurring protease is currently not known, cleavage occurs within the di-chain loop region between the two cysteine residues that form the disulfide bridge (see Table 3). Replacement of an endogenous protease cleavage site with an exogenous protease cleavage site will enable cleavage of a modified Clostridial toxin disclosed in the present specification when expressed in an organism that does not produce the naturally-occurring protease used to cleave the di-chain loop region of a toxin. Similarly, an addition of an exogenous protease cleavage site in the di-chain loop region will also enable cleavage of a modified Clostridial toxin disclosed in the present

specification when expressed in an organism that does not produce the naturally-occurring protease used to cleave the di-chain loop region of a toxin.

Table 4. Exogenous Protease Cleavage Sites

Protease Cleavage Site	Consensus Sequence	Non-limiting Examples	SEQ ID NO:
Bovine enterokinase	DDDDK*	DDDDK*	125
Tobacco Etch Virus (TEV)	E P^5 P^4YP^2Q*(G/S), where P^2, P^4 and P^5 can be any amino acid	ENLYFQ*G ENLYFQ*S ENIYTQ*G ENIYTQ*S ENIYLQ*G ENIYLQ*S ENVYFQ*G ENVYSQ*S ENVYSQ*G ENVYSQ*S	126 127 128 129 130 131 132 133 134 135
Human Rhinovirus 3C	P^5P^4LFQ*GP where P^4 is G, A, V, L, I, M, S or T and P^5 can any amino acid, with D or E preferred.	EALFQ*GP EVLFQ*GP ELLFQ*GP DALFQ*GP DVLFQ*GP DLLFQ*GP	136 137 138 139 140 141
SUMO/ULP-1	Tertiary structure	polypeptide-G*	142
Thrombin	P^3P^2(R/K)*$P^{1'}$, where P^3 is any amino acid and P^2 or $P^{1'}$ is G with the other position being any amino acid	GVR*G SAR*G SLR*G DGR*I QGK*I	143 144 145 146 147
Thrombin	P^4P^3P(R/K)*$P^{1'}P^{2'}$ where $P^{1'}$ and $P^{2'}$ can be any amino acid except for acidic amino acids like D or E; and P^3 and P^4 are hydrophobic amino acids like F, L, I, Y, W, V, M, P, C or A	LVPR*GS LVPK*GS FIPR*TF VLPR*SF IVPR*SF IVPR*GY VVPR*GV VLPR*LI VMPR*SL MFPR*SL	148 149 150 151 152 153 154 155 156 157
Coagulation Factor Xa	I(E/D)GR*	IDGR* IEGR*	158 159
An asterisks (*) indicates the peptide bond that is cleaved by the indicated protease.			

[0343] It is envisioned that an exogenous protease cleavage site of any and all lengths can be useful in aspects of the present invention with the proviso that the exogenous protease cleavage site is capable of being cleaved by its respective protease. Thus, in aspects of this embodiment, an exogenous protease cleavage site can be, *e.g.*, at least 6 amino acids in length, at least 7 amino acids in length, at least 8 amino acids in length, at least 9 amino acids in length, at least 10 amino acids in length, at least 15 amino acids in length, at least 20 amino acids in length, at least 25 amino acids in length, at least 30 amino

acids in length, at least 40 amino acids in length, at least 50 amino acids in length or at least 60 amino acids in length. In other aspects of this embodiment, an exogenous protease cleavage site can be, *e.g.*, at most 6 amino acids in length, at most 7 amino acids in length, at most 8 amino acids in length, at most 9 amino acids in length, at most 10 amino acids in length, at most 15 amino acids in length, at most 20 amino acids in length, at most 25 amino acids in length, at most 30 amino acids in length, at most 40 amino acids in length, at most 50 amino acids in length or at most 60 amino acids in length.

[0344] In aspects of this embodiment, a di-chain loop region can be modified to substitute a naturally-occurring protease cleavage site for an exogenous protease cleavage site. In this type of modification, the naturally-occurring protease cleavage site is made inoperable and thus can not be cleaved by its protease. Only the exogenous protease cleavage site can be cleaved by its corresponding exogenous protease. In this type of modification, the exogenous protease site is operably-linked in-frame to a modified Clostridial toxin as a fusion protein and the site can be cleaved by its respective exogenous protease. As a non-limiting example, a single-chain modified BoNT/A comprising an exogenous protease cleavage site in the di-chain loop region can be cleaved by its respective exogenous protease to produce the di-chain form of the toxin.

[0345] In other aspects of this embodiment, a di-chain loop region can be modified to include an exogenous protease cleavage site in addition to the naturally-occurring protease cleavage site. In this type of modification, both cleavage sites are operably-linked in-frame to a modified Clostridial toxin as a fusion protein and both sites can be cleaved by their respective proteases. As a non-limiting example, a single-chain modified BoNT/A that comprises a di-chain loop containing both the naturally-occurring BoNT/A di-chain loop protease cleavage site and an exogenous protease cleavage site can be cleaved by either the naturally occurring di-chain loop protease or by the appropriate exogenous protease to produce the di-chain form of the toxin.

[0346] A naturally-occurring protease cleavage site can be made inoperable by altering at least the two amino acids flanking the peptide bond cleaved by the naturally-occurring di-chain loop protease. More extensive alterations can be made, with the proviso that the two cysteine residues of the di-chain loop region remain intact and can still form the disulfide bridge. Non-limiting examples of an amino acid alteration include deletion of an amino acid or replacement of the original amino acid with a different amino acid. Thus, in one embodiment, a naturally-occurring protease cleavage site is made inoperable by altering the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease. In other aspects of this embodiment, a naturally-occurring protease cleavage site is made inoperable by altering, *e.g.*, at least three amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least four amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least five amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least six amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at

least seven amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least eight amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least nine amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least ten amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least 15 amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; or at least 20 amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease.

[0347] In still other aspects of this embodiment, a naturally-occurring di-chain protease cleavage site is made inoperable by altering, *e.g.*, at most three amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most four amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most five amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most six amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most seven amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most eight amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most nine amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most ten amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most 15 amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; or at most 20 amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease.

[0348] In an embodiment, an exogenous protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In aspects of this embodiment, a modified Clostridial toxin comprises an exogenous protease cleavage site comprises, *e.g.*, a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site. In other aspects of this embodiment, an exogenous protease cleavage site is located within the di-chain loop of, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0349] In an aspect of this embodiment, an exogenous protease cleavage site can be, *e.g.*, a bovine enterokinase cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can be, *e.g.*, a bovine enterokinase protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 125. Is still other aspects of this embodiment, a bovine enterokinase protease cleavage site is

located within the di-chain loop of, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0350] In another aspect of this embodiment, an exogenous protease cleavage site can be, *e.g.*, a Tobacco Etch Virus protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can be, *e.g.*, a Tobacco Etch Virus protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134 or SEQ ID NO: 135. Is still other aspects of this embodiment, a Tobacco Etch Virus protease cleavage site is located within the di-chain loop of, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0351] In still another aspect of this embodiment, an exogenous protease cleavage site can be, *e.g.*, a Human Rhinovirus 3C protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can be, *e.g.*, a Human Rhinovirus 3C protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140 or SEQ ID NO: 141. Is still other aspects of this embodiment, a Human Rhinovirus 3C protease cleavage site is located within the di-chain loop of, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0352] In yet another aspect of this embodiment, an exogenous protease cleavage site can be, *e.g.*, a SUMO/ULP-1 protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can be, *e.g.*, a SUMO/ULP-1 protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 142. Is still other aspects of this embodiment, a SUMO/ULP-1 protease cleavage site is located within the di-chain loop of, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0353] In a further aspect of this embodiment, an exogenous protease cleavage site can be, *e.g.*, a Thrombin protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can be, *e.g.*, a Thrombin protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156 or SEQ ID NO: 157. Is still other aspects of this embodiment, a Thrombin protease cleavage site is located within the di-chain loop of, *e.g.*, a modified BoNT/A, a modified BoNT/B,

a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0354] In another aspect of this embodiment, an exogenous protease cleavage site can be, *e.g.*, a Coagulation Factor Xa protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can be, *e.g.*, a Coagulation Factor Xa protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 158 or SEQ ID NO: 159. Is still other aspects of this embodiment, a Coagulation Factor Xa protease cleavage site is located within the di-chain loop of, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0355] In another embodiment, an exogenous protease site comprises a Clostridial toxin substrate cleavage site. As used herein, the term "Clostridial toxin substrate cleavage site" means a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by a Clostridial toxin under conditions suitable for Clostridial toxin protease activity. By definition, a Clostridial toxin substrate cleavage site is susceptible to cleavage by at least one Clostridial toxin under conditions suitable for Clostridial toxin protease activity. Non-limiting examples of Clostridial toxin substrate cleavage site are disclosed in, *e.g.*, Lance E. Steward et al., *Self-Activating Clostridial Toxins*, U.S. Patent Application 60/718,616 (Sep, 19, 2005).

[0356] It is understood that a modified Clostridial toxin disclosed in the present specification can optionally include one or more additional components. As a non-limiting example of an optional component, a modified Clostridial toxin can further comprise a flexible region comprising a flexible spacer. Non-limiting examples of a flexible spacer include, *e.g.*, a G-spacer GGGGS (SEQ ID NO: 160) or an A-spacer EAAAK (SEQ ID NO: 161). A flexible region comprising flexible spacers can be used to adjust the length of a polypeptide region in order to optimize a characteristic, attribute or property of a polypeptide. Such a flexible region is operably-linked in-frame to the modified Clostridial toxin as a fusion protein. As a non-limiting example, a polypeptide region comprising one or more flexible spacers in tandem can be use to better expose a protease cleavage site thereby facilitating cleavage of that site by a protease. As another non-limiting example, a polypeptide region comprising one or more flexible spacers in tandem can be use to better present an altered targeting domain, thereby facilitating the binding of that altered targeting domain to its receptor.

[0357] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise a flexible region comprising a flexible spacer. In another embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise flexible region comprising a plurality of flexible spacers in tandem. In aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at least 1 G-spacer, at least 2 G-spacers, at least 3 G-spacers, at least 4 G-spacers or at

least 5 G-spacers. In other aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at most 1 G-spacer, at most 2 G-spacers, at most 3 G-spacers, at most 4 G-spacers or at most 5 G-spacers. In still other aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at least 1 A-spacer, at least 2 A-spacers, at least 3 A-spacers, at least 4 A-spacers or at least 5 A-spacers. In still other aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at most 1 A-spacer, at most 2 A-spacers, at most 3 A-spacers, at most 4 A-spacers or at most 5 A-spacers. In another aspect of this embodiment, a modified Clostridial toxin can comprise a flexible region comprising one or more copies of the same flexible spacers, one or more copies of different flexible-spacer regions, or any combination thereof.

[0358] In aspects of this embodiment, a modified Clostridial toxin comprising a flexible spacer can be, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0359] It is envisioned that a modified Clostridial toxin disclosed in the present specification can comprise a flexible spacer in any and all locations with the proviso that modified Clostridial toxin is capable of performing the intoxication process. In aspects of this embodiment, a flexible spacer is positioned between, *e.g.*, an enzymatic domain and a translocation domain, an enzymatic domain and an altered targeting domain, an enzymatic domain and a protease cleavage site. In other aspects of this embodiment, a G-spacer is positioned between, *e.g.*, an enzymatic domain and a translocation domain, an enzymatic domain and an altered targeting domain, an enzymatic domain and a protease cleavage site. In other aspects of this embodiment, a A-spacer is positioned between, *e.g.*, an enzymatic domain and a translocation domain, an enzymatic domain and an altered targeting domain, an enzymatic domain and a protease cleavage site.

[0360] In other aspects of this embodiment, a flexible spacer is positioned between, *e.g.*, an altered targeting domain and a translocation domain, an altered targeting domain and an enzymatic domain, an altered targeting domain and a protease cleavage site. In other aspects of this embodiment, a G-spacer is positioned between, *e.g.*, an altered targeting domain and a translocation domain, an altered targeting domain and an enzymatic domain, an altered targeting domain and a protease cleavage site. In other aspects of this embodiment, a A-spacer is positioned between, *e.g.*, an altered targeting domain and a translocation domain, an altered targeting domain and an enzymatic domain, an altered targeting domain and a protease cleavage site.

[0361] In yet other aspects of this embodiment, a flexible spacer is positioned between, *e.g.*, a translocation domain and an enzymatic domain, an translocation domain and an altered targeting domain, an translocation domain and a protease cleavage site. In other aspects of this embodiment, a G-spacer is positioned between, *e.g.*, a translocation domain and an enzymatic domain, an translocation domain and an altered targeting domain, an translocation domain and a protease cleavage site. In other aspects

of this embodiment, a A-spacer is positioned between, *e.g.*, a translocation domain and an enzymatic domain, an translocation domain and an altered targeting domain, a translocation domain and a protease cleavage site.

[0362] As another non-limiting example of an optional component, a modified Clostridial toxin can further comprise an epitope-binding region. An epitope-binding region can be used in a wide variety of procedures involving, *e.g.*, protein purification and protein visualization. Such an epitope-binding region is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. Non-limiting examples of an epitope-binding region include, *e.g.*, FLAG, Express™ (SEQ ID NO: 162), human Influenza virus hemagluttinin (HA) (SEQ ID NO: 163), human $p62^{c\text{-}Myc}$ protein (c-MYC) (SEQ ID NO: 164), Vesicular Stomatitis Virus Glycoprotein (VSV-G) (SEQ ID NO: 165), Substance P (SEQ ID NO: 166), glycoprotein-D precursor of Herpes simplex virus (HSV) (SEQ ID NO: 167), V5 (SEQ ID NO: 168), AU1 (SEQ ID NO: 169) and AU5 (SEQ ID NO: 170); affinity-binding, such as. *e.g.*, polyhistidine (HIS) (SEQ ID NO: 171), streptavidin binding peptide (strep), and biotin or a biotinylation sequence; peptide-binding regions, such as. *e.g.*, the glutathione binding domain of glutathione-S-transferase, the calmodulin binding domain of the calmodulin binding protein, and the maltose binding domain of the maltose binding protein. Non-limiting examples of specific protocols for selecting, making and using an appropriate binding peptide are described in, *e.g.*, Epitope Tagging, pp. 17.90-17.93 (Sambrook and Russell, eds., Molecular Cloning A Laboratory Manual, Vol. 3, 3rd ed. 2001); Antibodies: A Laboratory Manual (Edward Harlow & David Lane, eds., Cold Spring Harbor Laboratory Press, 2nd ed. 1998); and Using Antibodies: A Laboratory Manual: Portable Protocol No. I (Edward Harlow & David Lane, Cold Spring Harbor Laboratory Press, 1998). In addition, non-limiting examples of binding peptides as well as well-characterized reagents, conditions and protocols are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[0363] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise an epitope-binding region. In another embodiment, a modified Clostridial toxin disclosed in the present specification can further comprises a plurality of epitope-binding regions. In aspects of this embodiment, a modified Clostridial toxin can comprise, *e.g.*, at least 1 epitope-binding region, at least 2 epitope-binding regions, at least 3 epitope-binding regions, at least 4 epitope-binding regions or at least 5 epitope-binding regions. In other aspects of this embodiment, a modified Clostridial toxin can comprise, *e.g.*, at most 1 epitope-binding region, at most 2 epitope-binding regions, at most 3 epitope-binding regions, at most 4 epitope-binding regions or at most 5 epitope-binding regions. In another aspect of this embodiment, a modified Clostridial toxin can comprise one or more copies of the same epitope-binding region, one or more copies of different epitope-binding regions, or any combination thereof.

[0364] The location of an epitope-binding region can be in various positions, including, without limitation, at the amino terminus of a modified Clostridial toxin, within a modified Clostridial toxin, or at the carboxyl terminus of a modified Clostridial toxin. Thus, in an embodiment, an epitope-binding region is located at the amino-terminus of a modified Clostridial toxin. In such a location, a start methionine should be placed in front of the epitope-binding region. In addition, it is known in the art that when adding a polypeptide that is operably-linked to the amino terminus of another polypeptide comprising the start methionine that the original methionine residue can be deleted. This is due to the fact that the added polypeptide will contain a new start methionine and that the original start methionine may reduce optimal expression of the fusion protein. In aspects of this embodiment, an epitope-binding region located at the amino-terminus of a modified Clostridial toxin disclosed in the present specification can be, *e.g.*, a FLAG, Express™ epitope-binding region, a human Influenza virus hemagluttinin (HA) epitope-binding region, a human $p62^{c\text{-}Myc}$ protein (c-MYC) epitope-binding region, a Vesicular Stomatitis Virus Glycoprotein (VSV-G) epitope-binding region, a Substance P epitope-binding region, a glycoprotein-D precursor of Herpes simplex virus (HSV) epitope-binding region, a V5 epitope-binding region, a AU1 epitope-binding region, a AU5 epitope-binding region, a polyhistidine epitope-binding region, a streptavidin binding peptide epitope-binding region, a biotin epitope-binding region, a biotinylation epitope-binding region, a glutathione binding domain of glutathione-S-transferase, a calmodulin binding domain of the calmodulin binding protein or a maltose binding domain of the maltose binding protein.

[0365] In another embodiment, an epitope-binding region is located at the carboxyl-terminus of a modified Clostridial toxin. In aspects of this embodiment, an epitope-binding region located at the carboxyl-terminus of a modified Clostridial toxin disclosed in the present specification can be, *e.g.*, a FLAG, Express™ epitope-binding region, a human Influenza virus hemagluttinin (HA) epitope-binding region, a human $p62^{c\text{-}Myc}$ protein (c-MYC) epitope-binding region, a Vesicular Stomatitis Virus Glycoprotein (VSV-G) epitope-binding region, a Substance P epitope-binding region, a glycoprotein-D precursor of Herpes simplex virus (HSV) epitope-binding region, a V5 epitope-binding region, a AU1 epitope-binding region, a AU5 epitope-binding region, a polyhistidine epitope-binding region, a streptavidin binding peptide epitope-binding region, a biotin epitope-binding region, a biotinylation epitope-binding region, a glutathione binding domain of glutathione-S-transferase, a calmodulin binding domain of the calmodulin binding protein or a maltose binding domain of the maltose binding protein.

[0366] It is envisioned that a modified Clostridial toxin disclosed in the present specification can comprise an altered targeting domain in any and all locations with the proviso that modified Clostridial toxin is capable of performing the intoxication process. Non-limiting examples include, locating an enhance targeting domain at the amino terminus of a modified Clostridial toxin (see FIG. 4); locating an enhance targeting domain between a Clostridial toxin enzymatic domain and a Clostridial toxin translocation domain of a modified Clostridial toxin (see FIG. 5); and locating an enhance targeting domain at the carboxyl terminus of a modified Clostridial toxin (see FIG. 6). The enzymatic domain of naturally-occurring Clostridial toxins contains the native start methionine. Thus, in domain organizations

where the enzymatic domain is not in the amino-terminal location an amino acid sequence comprising the start methionine should be placed in front of the amino-terminal domain. Likewise, where the altered targeting domain is in the amino-terminal position, an amino acid sequence comprising a start methionine and a protease cleavage site may be operably-linked in situations in which the altered targeting domain requires a free amino terminus, see, *e.g.*, Shengwen Li et al., *Degradable Clostridial Toxins*, International Patent Application Publication WO 2006/026780 (Mar. 9, 2006). In addition, it is known in the art that when adding a polypeptide that is operably-linked to the amino terminus of another polypeptide comprising the start methionine that the original methionine residue can be deleted.

[0367] Thus, in an embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a Clostridial toxin translocation facilitating domain and an altered targeting domain. In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, a protease cleavage site, a Clostridial toxin translocation domain, a Clostridial toxin translocation facilitating domain and an altered targeting domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an endogenous protease cleavage site, a Clostridial toxin translocation domain, a Clostridial toxin translocation facilitating domain and an altered targeting domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an exogenous protease cleavage site, a Clostridial toxin translocation domain, a Clostridial toxin translocation facilitating domain and an altered targeting domain.

[0368] In another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an altered targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin translocation facilitating domain. In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, a protease cleavage site, an altered targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin translocation facilitating domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an endogenous protease cleavage site, an altered targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin translocation facilitating domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an exogenous protease cleavage site, an altered targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin translocation facilitating domain.

[0369] In another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an altered targeting domain, a Clostridial toxin translocation domain,

a Clostridial toxin translocation facilitating domain and a Clostridial toxin enzymatic domain. In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an altered targeting domain, a Clostridial toxin translocation domain, a Clostridial toxin translocation facilitating domain, a protease cleavage site and a Clostridial toxin enzymatic domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an altered targeting domain, a Clostridial toxin translocation domain, a Clostridial toxin translocation facilitating domain, an endogenous protease cleavage site and a Clostridial toxin enzymatic domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an altered targeting domain, a Clostridial toxin translocation domain, a Clostridial toxin translocation facilitating domain, an exogenous protease cleavage site and a Clostridial toxin enzymatic domain.

[0370] In another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an altered targeting domain, a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and a Clostridial toxin translocation facilitating domain. In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an altered targeting domain, a Clostridial toxin enzymatic domain, a protease cleavage site, a Clostridial toxin translocation domain and a Clostridial toxin translocation facilitating domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an altered targeting domain, a Clostridial toxin enzymatic domain, an endogenous protease cleavage site, a Clostridial toxin translocation domain and a Clostridial toxin translocation facilitating domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an altered targeting domain, a Clostridial toxin enzymatic domain, an exogenous protease cleavage site, a Clostridial toxin translocation domain and a Clostridial toxin translocation facilitating domain.

[0371] In another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, a Clostridial toxin translocation facilitating domain, a Clostridial toxin enzymatic domain and an altered targeting domain. In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, a Clostridial toxin translocation facilitating domain, a protease cleavage site, a Clostridial toxin enzymatic domain and an altered targeting domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, a Clostridial toxin translocation facilitating domain, an endogenous protease cleavage site, a Clostridial toxin enzymatic domain and an altered targeting domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, a Clostridial toxin translocation facilitating domain, an exogenous protease cleavage site, a Clostridial toxin enzymatic domain and an altered targeting domain.

[0372] Aspects of the present invention provide, in part modified Clostridial toxins. Non-limiting examples of Clostridial toxin modifications disclosed in the present specification include, *e.g.*, addition of an altered targeting domain, addition of a protease cleavage site, rearrangement of the enzymatic, translocation and binding domains and addition of a spacer region. It is understood that all such modifications do not substantially affect the ability of a modified Clostridial toxin to intoxicate a cell. As used herein, the term "do not substantially affect" means a modified Clostridial toxin can still execute the overall cellular mechanism whereby a Clostridial toxin enters a neuron and inhibits neurotransmitter release and encompasses the binding of a Clostridial toxin to a low or high affinity receptor complex, the internalization of the toxin/receptor complex, the translocation of the Clostridial toxin light chain into the cytoplasm and the enzymatic modification of a Clostridial toxin substrate. In aspects of this embodiment, the modified Clostridial toxin is, *e.g.*, at least 10% as toxic as a naturally-occurring Clostridial toxin, at least 20% as toxic as a naturally-occurring Clostridial toxin, at least 30% as toxic as a naturally-occurring Clostridial toxin, at least 40% as toxic as a naturally-occurring Clostridial toxin, at least 50% as toxic as a naturally-occurring Clostridial toxin, at least 60% as toxic as a naturally-occurring Clostridial toxin, at least 70% as toxic as a naturally-occurring Clostridial toxin, at least 80% as toxic as a naturally-occurring Clostridial toxin, at least 90% as toxic as a naturally-occurring Clostridial toxin or at least 95% as toxic as a naturally-occurring Clostridial toxin. In aspects of this embodiment, the modified Clostridial toxin is, *e.g.*, at most 10% as toxic as a naturally-occurring Clostridial toxin, at most 20% as toxic as a naturally-occurring Clostridial toxin, at most 30% as toxic as a naturally-occurring Clostridial toxin, at most 40% as toxic as a naturally-occurring Clostridial toxin, at most 50% as toxic as a naturally-occurring Clostridial toxin, at most 60% as toxic as a naturally-occurring Clostridial toxin, at most 70% as toxic as a naturally-occurring Clostridial toxin, at most 80% as toxic as a naturally-occurring Clostridial toxin, at most 90% as toxic as a naturally-occurring Clostridial toxin or at most 95% as toxic as a naturally-occurring Clostridial toxin.

[0373] Another aspect of the present invention provides polynucleotide molecules encoding modified Clostridial toxins disclosed in the present specification. It is envisioned that any and all modified Clostridial toxin disclosed in the present specification can be encoded by a polynucleotide molecule.

[0374] Aspects of the present invention provide, in part polynucleotide molecules. As used herein, the term "polynucleotide molecule" is synonymous with "nucleic acid molecule" and means a polymeric form of nucleotides, such as, *e.g.*, ribonucleotides and deoxyribonucleotides, of any length. It is envisioned that any and all polynucleotide molecules that can encode a modified Clostridial toxin disclosed in the present specification can be useful, including, without limitation naturally-occurring and non-naturally-occurring DNA molecules and naturally-occurring and non-naturally-occurring RNA molecules. Non-limiting examples of naturally-occurring and non-naturally-occurring DNA molecules include single-stranded DNA molecules, double-stranded DNA molecules, genomic DNA molecules, cDNA molecules, vector constructs, such as, *e.g.*, plasmid constructs, phagmid constructs, bacteriophage constructs,

retroviral constructs and artificial chromosome constructs. Non-limiting examples of naturally-occurring and non-naturally-occurring RNA molecules include single-stranded RNA, double stranded RNA and mRNA.

[0375] Thus, in an embodiment, a polynucleotide molecule encodes a modified Clostridial toxin disclosed in the present specification.

[0376] In another embodiment, a polynucleotide molecule encodes, in part, a modified Clostridial toxin comprising a Clostridial toxin enzymatic domain disclosed in the present specification. In an aspect of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin enzymatic domain comprises a naturally occurring Clostridial toxin enzymatic domain variant, such as, *e.g.*, a Clostridial toxin enzymatic domain isoform or a Clostridial toxin enzymatic domain subtype. In another aspect of this embodiment, a polynucleotide molecule encoding a Clostridial toxin enzymatic domain comprises a non-naturally occurring Clostridial toxin enzymatic domain variant, such as, *e.g.*, a conservative Clostridial toxin enzymatic domain variant, a non-conservative Clostridial toxin enzymatic domain variant or an active Clostridial toxin enzymatic domain fragment, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encoding a Clostridial toxin enzymatic domain comprises a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a TeNT enzymatic domain, or active fragment thereof.

[0377] In another embodiment, a polynucleotide molecule encodes, in part, a modified Clostridial toxin comprising a Clostridial toxin translocation domain disclosed in the present specification. In an aspect of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin translocation domain comprises a naturally occurring Clostridial toxin translocation domain variant, such as, *e.g.*, a Clostridial toxin translocation domain isoform or a Clostridial toxin translocation domain subtype. In another aspect of this embodiment, a polynucleotide molecule encoding a Clostridial toxin translocation domain comprises a non-naturally occurring Clostridial toxin translocation domain variant, such as, *e.g.*, a conservative Clostridial toxin translocation domain variant, a non-conservative Clostridial toxin translocation domain variant or an active Clostridial toxin translocation domain fragment, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encoding a Clostridial toxin translocation domain comprises a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, or active fragment thereof.

[0378] In another embodiment, a polynucleotide molecule encodes, in part, a modified Clostridial toxin comprising a translocation facilitating domain disclosed in the present specification. In an aspect of this embodiment, a polynucleotide molecule encoding a translocation facilitating domain comprises a naturally

occurring Clostridial toxin translocation facilitating domain variant, such as, *e.g.*, a Clostridial toxin translocation facilitating domain isoform or a Clostridial toxin translocation facilitating domain subtype. In another aspect of this embodiment, a polynucleotide molecule encoding a translocation facilitating domain comprises a non-naturally occurring Clostridial toxin translocation facilitating domain variant, such as, *e.g.*, a conservative Clostridial toxin translocation facilitating domain variant, a non-conservative Clostridial toxin translocation facilitating domain variant or an active Clostridial toxin translocation facilitating domain fragment, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encoding a Clostridial toxin translocation facilitating domain comprises a BoNT/A translocation facilitating domain, a BoNT/B translocation facilitating domain, a BoNT/C1 translocation facilitating domain, a BoNT/D translocation facilitating domain, a BoNT/E translocation facilitating domain, a BoNT/F translocation facilitating domain, a BoNT/G translocation facilitating domain, a TeNT translocation facilitating domain, or active fragment thereof. In yet another aspect of this embodiment, a polynucleotide molecule encoding a translocation domain comprises a naturally occurring enveloped virus fusogenic peptide domain variant, such as, *e.g.*, an enveloped virus fusogenic peptide domain isoform or an enveloped virus fusogenic peptide domain subtype. In another aspect of this embodiment, a polynucleotide molecule encoding a translocation domain comprises a non-naturally occurring enveloped virus fusogenic peptide domain variant, such as, *e.g.*, a conservative enveloped virus fusogenic peptide domain variant, a non-conservative enveloped virus fusogenic peptide domain variant or an active enveloped virus fusogenic peptide domain fragment, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encoding an enveloped virus fusogenic peptide domain comprisesan influenzavirus fusogenic peptide domain, an alphavirus fusogenic peptide domain, a vesiculovirus fusogenic peptide domain, a respirovirus fusogenic peptide domain, a morbillivirus fusogenic peptide domain, an avulavirus fusogenic peptide domain, a henipavirus fusogenic peptide domain, a metapneumovirus fusogenic peptide domain, a foamy virus fusogenic peptide domain, or active fragment thereof.

[0379] In another embodiment, a polynucleotide molecule encodes, in part, a modified Clostridial toxin comprising an altered targeting domain disclosed in the present specification. In an aspect of this embodiment, a polynucleotide molecule encoding an altered targeting domain comprises a polypeptide that selectively binds to a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell. In an aspect of this embodiment, a polynucleotide molecule encoding a polypeptide that selectively binds to a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell comprises a naturally occurring variant, such as, *e.g.*, an isoform or a subtype. In another aspect of this embodiment, a polynucleotide molecule encoding a polypeptide that selectively binds to a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell comprises a non-naturally occurring variant, such as, *e.g.*, a conservative variant, a non-conservative variant or an active fragment, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encoding a polypeptide that selectively binds to a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell comprises an opioid peptide, such as, *e.g.*, an enkephalin, a bovine adrenomedullary-22 (BAM22) peptide, an endomorphin, an endorphin, a

dynorphin, a nociceptin or a hemorphin; a melanocortin peptide, such as, *e.g.*, an α-melanocyte stimulating hormones (α-MSH), a β-melanocyte stimulating hormones (β-MSH), a γ-melanocyte stimulating hormones (γ-MSH), an adrenocorticotropin (ACTH), a Corticotropin-like intermediary peptide (CLIP), a β-lipotropin (β-LPH) and a γ-lipotropin (γ-LPH); a galanin, such as, *e.g.*, a galanin and a galanin message-associated peptide (GMAP); a granin, such as, *e.g.*, a chromogranin A peptide like a β-granin, a vasostatin, a chromostatin, a pancreastatin, a WE-14, a catestatin, a parastatin and a GE-25, a chromogranin B (secretogranin I) peptide like a GAWK peptide, a adrenomedullary peptide and a secretolytin and a chromogranin C (secretogranin II) peptide like secretoneurin, EM66 and manserin; a tachykinin peptide, such as, *e.g.*, Substance P, neuropeptide K (NPK), neuropeptide gamma (NP gamma), neurokinin A (NKA; Substance K, neurokinin alpha, neuromedin L), neurokinin B (NKB), a hemokinin and a endokinin; a cholecystokinin, such as, *e.g.*, a cholecystokinin 58, a cholecystokinin 39, a cholecystokinin 33, a cholecystokinin 12 and a cholecystokinin 8; a Neuropeptide Y related peptide, such as, *e.g.*, a Neuropeptide Y (NPY), a Peptide YY (PYY), Pancreatic peptide (PP) and a Pancreatic icosapeptide (PIP); , a kinin peptide, such as, *e.g.*, a bradykinin, a kallidan, a desArg^{9} bradykinin and a desArg^{10} bradykinin; a protease activated receptor (PAR) peptide, such as, *e.g.*, a PAR1 peptide, a PAR2 peptide, a PAR3 peptide and a PAR4 peptide; a corticotropin-releasing hormone; a thyrotropin-releasing hormone; a somatostatin; a leukemia inhibitor factor (LIF); and an interleukin-1 (IL1).

[0380] In another embodiment, a polynucleotide molecule encodes, in part, a modified Clostridial toxin comprising a protease cleavage site disclosed in the present specification. In an aspect of this embodiment, a polynucleotide molecule encoding a protease cleavage site comprises an endogenous Clostridial toxin protease site. In aspects of this embodiment, a polynucleotide molecule encoding an endogenous Clostridial toxin protease site can be, *e.g.*, a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site or a TeNT di-chain loop protease cleavage site. In another aspect of this embodiment, a polynucleotide molecule encoding a protease cleavage site comprises an exogenous Clostridial toxin protease site. In aspects of this embodiment, a polynucleotide molecule encoding an exogenous Clostridial toxin protease site can be, *e.g.*, a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site, a Coagulation Factor Xa protease cleavage site or a Clostridial toxin substrate cleavage site, such as, *e.g.*, a BoNT/A substrate cleavage site, a BoNT/B substrate cleavage site, a BoNT/C1 substrate cleavage site, a BoNT/D substrate cleavage site, a BoNT/E substrate cleavage site, a BoNT/F substrate cleavage site, a BoNT/G substrate cleavage site or a TeNT substrate cleavage site.

[0381] In another embodiment, a polynucleotide molecule encodes, in part, a modified Clostridial toxin comprising a flexible spacer disclosed in the present specification. In an aspect of this embodiment, a polynucleotide molecule encoding a flexible spacer a G-spacer, a A-spacer of any combination thereof.

[0382] Well-established molecular biology techniques that may be necessary to make a polynucleotide molecule encoding a modified Clostridial toxin disclosed in the present specification including, but not limited to, procedures involving polymerase chain reaction (PCR) amplification, restriction enzyme reactions, agarose gel electrophoresis, nucleic acid ligation, bacterial transformation, nucleic acid purification, nucleic acid sequencing and recombination-based techniques are routine procedures well within the scope of one skilled in the art and from the teaching herein. Non-limiting examples of specific protocols necessary to make a polynucleotide molecule encoding a modified Clostridial toxin are described in *e.g.*, MOLECULAR CLONING A LABORATORY MANUAL, *supra*, (2001); and CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Frederick M. Ausubel et al., eds. John Wiley & Sons, 2004). Additionally, a variety of commercially available products useful for making a polynucleotide molecule encoding a modified Clostridial toxin are widely available. These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[0383] Another aspect of the present invention provides a method of producing a modified Clostridial toxin disclosed in the present specification, such method comprising the step of expressing a polynucleotide molecule encoding a modified Clostridial toxin in a cell. Another aspect of the present invention provides a method of producing a modified Clostridial toxin disclosed in the present specification, such method comprising the steps of introducing an expression construct comprising a polynucleotide molecule encoding a modified Clostridial toxin into a cell and expressing the expression construct in the cell.

[0384] The methods disclosed in the present specification include, in part, a modified Clostridial toxin. It is envisioned that any and all modified Clostridial toxins disclosed in the present specification can be produced using the methods disclosed in the present specification. It is also envisioned that any and all polynucleotide molecules encoding a modified Clostridial toxins disclosed in the present specification can be useful in producing a modified Clostridial toxins disclosed in the present specification using the methods disclosed in the present specification.

[0385] The methods disclosed in the present specification include, in part, an expression construct. An expression construct comprises a polynucleotide molecule disclosed in the present specification operably-linked to an expression vector useful for expressing the polynucleotide molecule in a cell or cell-free extract. A wide variety of expression vectors can be employed for expressing a polynucleotide molecule encoding a modified Clostridial toxin, including, without limitation, a viral expression vector; a prokaryotic expression vector; eukaryotic expression vectors, such as, *e.g.*, a yeast expression vector, an insect expression vector and a mammalian expression vector; and a cell-free extract expression vector. It is further understood that expression vectors useful to practice aspects of these methods may include those which express a modified Clostridial toxin under control of a constitutive, tissue-specific, cell-specific or inducible promoter element, enhancer element or both. Non-limiting examples of expression

vectors, along with well-established reagents and conditions for making and using an expression construct from such expression vectors are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; EMD Biosciences-Novagen, Madison, WI; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. The selection, making and use of an appropriate expression vector are routine procedures well within the scope of one skilled in the art and from the teachings herein.

[0386] Thus, aspects of this embodiment include, without limitation, a viral expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; a prokaryotic expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; a yeast expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; an insect expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; and a mammalian expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin. Other aspects of this embodiment include, without limitation, expression constructs suitable for expressing a modified Clostridial toxin disclosed in the present specification using a cell-free extract comprising a cell-free extract expression vector operably linked to a polynucleotide molecule encoding a modified Clostridial toxin.

[0387] The methods disclosed in the present specification include, in part, a cell. It is envisioned that any and all cells can be used. Thus, aspects of this embodiment include, without limitation, prokaryotic cells including, without limitation, strains of aerobic, microaerophilic, capnophilic, facultative, anaerobic, gram-negative and gram-positive bacterial cells such as those derived from, *e.g.*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacteroides fragilis*, *Clostridia perfringens*, *Clostridia* difficile, *Caulobacter crescentus*, *Lactococcus lactis*, *Methylobacterium extorquens*, *Neisseria meningirulls*, *Neisseria meningitidis*, *Pseudomonas fluorescens* and *Salmonella typhimurium*; and eukaryotic cells including, without limitation, yeast strains, such as, *e.g.*, those derived from *Pichia pastoris*, *Pichia methanolica*, *Pichia angusta*, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica*; insect cells and cell lines derived from insects, such as, *e.g.*, those derived from *Spodoptera frugiperda*, *Trichoplusia ni*, *Drosophila melanogaster* and *Manduca sexta*; and mammalian cells and cell lines derived from mammalian cells, such as, *e.g.*, those derived from mouse, rat, hamster, porcine, bovine, equine, primate and human. Cell lines may be obtained from the American Type Culture Collection, European Collection of Cell Cultures and the German Collection of Microorganisms and Cell Cultures. Non-limiting examples of specific protocols for selecting, making and using an appropriate cell line are described in *e.g.*, INSECT CELL CULTURE ENGINEERING (Mattheus F. A. Goosen et al. eds., Marcel Dekker, 1993); INSECT CELL CULTURES: FUNDAMENTAL AND APPLIED ASPECTS (J. M. Vlak et al. eds., Kluwer Academic Publishers, 1996); Maureen A. Harrison & Ian F. Rae, GENERAL TECHNIQUES OF CELL CULTURE (Cambridge University Press, 1997); CELL AND TISSUE CULTURE: LABORATORY PROCEDURES (Alan Doyle et al eds., John Wiley and Sons, 1998); R. Ian Freshney, CULTURE OF ANIMAL CELLS: A MANUAL OF BASIC TECHNIQUE (Wiley-Liss, 4th ed. 2000); ANIMAL CELL CULTURE: A PRACTICAL APPROACH (John R. W. Masters

ed., Oxford University Press, 3rd ed. 2000); MOLECULAR CLONING A LABORATORY MANUAL, *supra*, (2001); BASIC CELL CULTURE: A PRACTICAL APPROACH (John M. Davis, Oxford Press, 2nd ed. 2002); and CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, *supra*, (2004). These protocols are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0388] The methods disclosed in the present specification include, in part, introducing into a cell a polynucleotide molecule. A polynucleotide molecule introduced into a cell can be transiently or stably maintained by that cell. Stably-maintained polynucleotide molecules may be extra-chromosomal and replicate autonomously, or they may be integrated into the chromosomal material of the cell and replicate non-autonomously. It is envisioned that any and all methods for introducing a polynucleotide molecule disclosed in the present specification into a cell can be used. Methods useful for introducing a nucleic acid molecule into a cell include, without limitation, chemical-mediated transfection such as, *e.g.*, calcium phosphate-mediated, diethyl-aminoethyl (DEAE) dextran-mediated, lipid-mediated, polyethyleneimine (PEI)-mediated, polylysine-mediated and polybrene-mediated; physical-mediated tranfection, such as, *e.g.*, biolistic particle delivery, microinjection, protoplast fusion and electroporation; and viral-mediated transfection, such as, *e.g.*, retroviral-mediated transfection, see, *e.g.*, Introducing Cloned Genes into Cultured Mammalian Cells, pp. 16.1-16.62 (Sambrook & Russell, eds., Molecular Cloning A Laboratory Manual, Vol. 3, 3rd ed. 2001). One skilled in the art understands that selection of a specific method to introduce an expression construct into a cell will depend, in part, on whether the cell will transiently contain an expression construct or whether the cell will stably contain an expression construct. These protocols are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0389] In an aspect of this embodiment, a chemical-mediated method, termed transfection, is used to introduce a polynucleotide molecule encoding a modified Clostridial toxin into a cell. In chemical-mediated methods of transfection the chemical reagent forms a complex with the nucleic acid that facilitates its uptake into the cells. Such chemical reagents include, without limitation, calcium phosphate-mediated, see, *e.g.*, Martin Jordan & Florian Worm, Transfection of adherent and suspended cells by calcium phosphate, 33(2) Methods 136-143 (2004); diethyl-aminoethyl (DEAE) dextran-mediated, lipid-mediated, cationic polymer-mediated like polyethyleneimine (PEI)-mediated and polylysine-mediated and polybrene-mediated, see, *e.g.*, Chun Zhang et al., Polyethylenimine strategies for plasmid delivery to brain-derived cells, 33(2) Methods 144-150 (2004). Such chemical-mediated delivery systems can be prepared by standard methods and are commercially available, see, *e.g.*, CellPhect Transfection Kit (Amersham Biosciences, Piscataway, NJ); Mammalian Transfection Kit, Calcium phosphate and DEAE Dextran, (Stratagene, Inc., La Jolla, CA); Lipofectamine™ Transfection Reagent (Invitrogen, Inc., Carlsbad, CA); ExGen 500 Transfection kit (Fermentas, Inc., Hanover, MD), and SuperFect and Effectene Transfection Kits (Qiagen, Inc., Valencia, CA).

[0390] In another aspect of this embodiment, a physical-mediated method is used to introduce a polynucleotide molecule encoding a modified Clostridial toxin into a cell. Physical techniques include,

without limitation, electroporation, biolistic and microinjection. Biolistics and microinjection techniques perforate the cell wall in order to introduce the nucleic acid molecule into the cell, see, *e.g.*, Jeike E. Biewenga et al., Plasmid-mediated gene transfer in neurons using the biolistics technique, 71(1) J. Neurosci. Methods. 67-75 (1997); and John O'Brien & Sarah C. R. Lummis, Biolistic and diolistic transfection: using the gene gun to deliver DNA and lipophilic dyes into mammalian cells, 33(2) Methods 121-125 (2004). Electroporation, also termed electropermeabilization, uses brief, high-voltage, electrical pulses to create transient pores in the membrane through which the nucleic acid molecules enter and can be used effectively for stable and transient transfections of all cell types, see, *e.g.*, M. Golzio et al., In vitro and in vivo electric field-mediated permeabilization, gene transfer, and expression, 33(2) Methods 126-135 (2004); and Oliver Greschet al., New non-viral method for gene transfer into primary cells, 33(2) Methods 151-163 (2004).

[0391] In another aspect of this embodiment, a viral-mediated method, termed transduction, is used to introduce a polynucleotide molecule encoding a modified Clostridial toxin into a cell. In viral-mediated methods of transient transduction, the process by which viral particles infect and replicate in a host cell has been manipulated in order to use this mechanism to introduce a nucleic acid molecule into the cell. Viral-mediated methods have been developed from a wide variety of viruses including, without limitation, retroviruses, adenoviruses, adeno-associated viruses, herpes simplex viruses, picornaviruses, alphaviruses and baculoviruses, see, *e.g.*, Armin Blesch, Lentiviral and MLV based retroviral vectors for ex vivo and in vivo gene transfer, 33(2) Methods 164-172 (2004); and Maurizio Federico, From lentiviruses to lentivirus vectors, 229 Methods Mol. Biol. 3-15 (2003); E. M. Poeschla, Non-primate lentiviral vectors, 5(5) Curr. Opin. Mol. Ther. 529-540 (2003); Karim Benihoud et al, Adenovirus vectors for gene delivery, 10(5) Curr. Opin. Biotechnol. 440-447 (1999); H. Bueler, Adeno-associated viral vectors for gene transfer and gene therapy, 380(6) Biol. Chem. 613-622 (1999); Chooi M. Lai et al., Adenovirus and adeno-associated virus vectors, 21(12) DNA Cell Biol. 895-913 (2002); Edward A. Burton et al., Gene delivery using herpes simplex virus vectors, 21(12) DNA Cell Biol. 915-936 (2002); Paola Grandi et al., Targeting HSV amplicon vectors, 33(2) Methods 179-186 (2004); Ilya Frolov et al., Alphavirus-based expression vectors: strategies and applications, 93(21) Proc. Natl. Acad. Sci. U. S. A. 11371-11377 (1996); Markus U. Ehrengruber, Alphaviral gene transfer in neurobiology, 59(1) Brain Res. Bull. 13-22 (2002); Thomas A. Kost & J. Patrick Condreay, Recombinant baculoviruses as mammalian cell gene-delivery vectors, 20(4) Trends Biotechnol. 173-180 (2002); and A. Huser & C. Hofmann, Baculovirus vectors: novel mammalian cell gene-delivery vehicles and their applications, 3(1) Am. J. Pharmacogenomics 53-63 (2003).

[0392] Adenoviruses, which are non-enveloped, double-stranded DNA viruses, are often selected for mammalian cell transduction because adenoviruses handle relatively large polynucleotide molecules of about 36 kb, are produced at high titer, and can efficiently infect a wide variety of both dividing and non-dividing cells, see, *e.g.*, Wim T. J. M. C. Hermens et al., Transient gene transfer to neurons and glia: analysis of adenoviral vector performance in the CNS and PNS, 71(1) J. Neurosci. Methods 85-98 (1997);

and Hiroyuki Mizuguchi et al., Approaches for generating recombinant adenovirus vectors, 52(3) Adv. Drug Deliv. Rev. 165-176 (2001). Transduction using adenoviral-based system do not support prolonged protein expression because the nucleic acid molecule is carried from an episome in the cell nucleus, rather than being integrated into the host cell chromosome. Adenoviral vector systems and specific protocols for how to use such vectors are disclosed in, *e.g.*, ViraPower™ Adenoviral Expression System (Invitrogen, Inc., Carlsbad, CA) and ViraPower™ Adenoviral Expression System Instruction Manual 25-0543 version A, Invitrogen, Inc., (Jul. 15, 2002); and AdEasy™ Adenoviral Vector System (Stratagene, Inc., La Jolla, CA) and AdEasy™ Adenoviral Vector System Instruction Manual 064004f, Stratagene, Inc..

[0393] Nucleic acid molecule delivery can also use single-stranded RNA retroviruses, such as, *e.g.*, oncoretroviruses and lentiviruses. Retroviral-mediated transduction often produce transduction efficiencies close to 100%, can easily control the proviral copy number by varying the multiplicity of infection (MOI), and can be used to either transiently or stably transduce cells, see, *e.g.*, Tiziana Tonini et al., Transient production of retroviral- and lentiviral-based vectors for the transduction of Mammalian cells, 285 Methods Mol. Biol. 141-148 (2004); Armin Blesch, Lentiviral and MLV based retroviral vectors for ex vivo and in vivo gene transfer, 33(2) Methods 164-172 (2004); Félix Recillas-Targa, Gene transfer and expression in mammalian cell lines and transgenic animals, 267 Methods Mol. Biol. 417-433 (2004); and Roland Wolkowicz et al., Lentiviral vectors for the delivery of DNA into mammalian cells, 246 Methods Mol. Biol. 391-411 (2004). Retroviral particles consist of an RNA genome packaged in a protein capsid, surrounded by a lipid envelope. The retrovirus infects a host cell by injecting its RNA into the cytoplasm along with the reverse transcriptase enzyme. The RNA template is then reverse transcribed into a linear, double stranded cDNA that replicates itself by integrating into the host cell genome. Viral particles are spread both vertically (from parent cell to daughter cells via the provirus) as well as horizontally (from cell to cell via virions). This replication strategy enables long-term persistent expression since the nucleic acid molecules of interest are stably integrated into a chromosome of the host cell, thereby enabling long-term expression of the protein. For instance, animal studies have shown that lentiviral vectors injected into a variety of tissues produced sustained protein expression for more than 1 year, see, *e.g.*, Luigi Naldini et al., In vivo gene delivery and stable transduction of non-dividing cells by a lentiviral vector, 272(5259) Science 263-267 (1996). The Oncoretroviruses-derived vector systems, such as, *e.g.*, Moloney murine leukemia virus (MoMLV), are widely used and infect many different non-dividing cells. Lentiviruses can also infect many different cell types, including dividing and non-dividing cells and possess complex envelope proteins, which allows for highly specific cellular targeting.

[0394] Retroviral vectors and specific protocols for how to use such vectors are disclosed in, *e.g.*, U.S. Patent Nos. Manfred Gossen & Hermann Bujard, Tight control of gene expression in eukaryotic cells by tetracycline-responsive promoters, U.S. Patent No. 5,464,758 (Nov. 7, 1995) and Hermann Bujard & Manfred Gossen, Methods for regulating gene expression, U.S. Patent No. 5,814,618 (Sep. 29, 1998) David S. Hogness, Polynucleotides encoding insect steroid hormone receptor polypeptides and cells transformed with same, U.S. Patent No. 5,514,578 (May 7, 1996) and David S. Hogness, Polynucleotide

encoding insect ecdysone receptor, U.S. Patent 6,245,531 (Jun. 12, 2001); Elisabetta Vegeto et al., Progesterone receptor having C. terminal hormone binding domain truncations, U.S. Patent No. 5,364,791 (Nov. 15, 1994), Elisabetta Vegeto et al., Mutated steroid hormone receptors, methods for their use and molecular switch for gene therapy, U.S. Patent No. 5,874,534 (Feb. 23, 1999) and Elisabetta Vegeto et al., Mutated steroid hormone receptors, methods for their use and molecular switch for gene therapy, U.S. Patent No. 5,935,934 (Aug. 10, 1999). Furthermore, such viral delivery systems can be prepared by standard methods and are commercially available, see, *e.g.*, BD™ Tet-Off and Tet-On Gene Expression Systems (BD Biosciences-Clonetech, Palo Alto, CA) and BD™ Tet-Off and Tet-On Gene Expression Systems User Manual, PT3001-1, BD Biosciences Clonetech, (Mar. 14, 2003), GeneSwitch™ System (Invitrogen, Inc., Carlsbad, CA) and GeneSwitch™ System A Mifepristone-Regulated Expression System for Mammalian Cells version D, 25-0313, Invitrogen, Inc., (Nov. 4, 2002); ViraPower™ Lentiviral Expression System (Invitrogen, Inc., Carlsbad, CA) and ViraPower™ Lentiviral Expression System Instruction Manual 25-0501 version E, Invitrogen, Inc., (Dec. 8, 2003); and Complete Control® Retroviral Inducible Mammalian Expression System (Stratagene, La Jolla, CA) and Complete Control® Retroviral Inducible Mammalian Expression System Instruction Manual, 064005e.

[0395] The methods disclosed in the present specification include, in part, expressing a modified Clostridial toxin from a polynucleotide molecule. It is envisioned that any of a variety of expression systems may be useful for expressing a modified Clostridial toxin from a polynucleotide molecule disclosed in the present specification, including, without limitation, cell-based systems and cell-free expression systems. Cell-based systems include, without limitation, viral expression systems, prokaryotic expression systems, yeast expression systems, baculoviral expression systems, insect expression systems and mammalian expression systems. Cell-free systems include, without limitation, wheat germ extracts, rabbit reticulocyte extracts and *E. coli* extracts and generally are equivalent to the method disclosed herein. Expression of a polynucleotide molecule using an expression system can include any of a variety of characteristics including, without limitation, inducible expression, non-inducible expression, constitutive expression, viral-mediated expression, stably-integrated expression, and transient expression. Expression systems that include well-characterized vectors, reagents, conditions and cells are well-established and are readily available from commercial vendors that include, without limitation, Ambion, Inc. Austin, TX; BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; QIAGEN, Inc., Valencia, CA; Roche Applied Science, Indianapolis, IN; and Stratagene, La Jolla, CA. Non-limiting examples on the selection and use of appropriate heterologous expression systems are described in *e.g.*, PROTEIN EXPRESSION. A PRACTICAL APPROACH (S. J. Higgins and B. David Hames eds., Oxford University Press, 1999); Joseph M. Fernandez & James P. Hoeffler, GENE EXPRESSION SYSTEMS. USING NATURE FOR THE ART OF EXPRESSION (Academic Press, 1999); and Meena Rai & Harish Padh, *Expression Systems for Production of Heterologous Proteins*, 80(9) CURRENT SCIENCE 1121-1128, (2001). These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[0396] A variety of cell-based expression procedures are useful for expressing a modified Clostridial toxin encoded by polynucleotide molecule disclosed in the present specification. Examples included, without limitation, viral expression systems, prokaryotic expression systems, yeast expression systems, baculoviral expression systems, insect expression systems and mammalian expression systems. Viral expression systems include, without limitation, the ViraPower™ Lentiviral (Invitrogen, Inc., Carlsbad, CA), the Adenoviral Expression Systems (Invitrogen, Inc., Carlsbad, CA), the AdEasy™ XL Adenoviral Vector System (Stratagene, La Jolla, CA) and the ViraPort® Retroviral Gene Expression System (Stratagene, La Jolla, CA). Non-limiting examples of prokaryotic expression systems include the Champion™ pET Expression System (EMD Biosciences-Novagen, Madison, WI), the TriEx™ Bacterial Expression System (EMD Biosciences-Novagen, Madison, WI), the QIA*express*® Expression System (QIAGEN, Inc.), and the Affinity® Protein Expression and Purification System (Stratagene, La Jolla, CA). Yeast expression systems include, without limitation, the EasySelect™ *Pichia* Expression Kit (Invitrogen, Inc., Carlsbad, CA), the YES-Echo™ Expression Vector Kits (Invitrogen, Inc., Carlsbad, CA) and the SpECTRA™ *S. pombe* Expression System (Invitrogen, Inc., Carlsbad, CA). Non-limiting examples of baculoviral expression systems include the BaculoDirect™ (Invitrogen, Inc., Carlsbad, CA), the Bac-to-Bac® (Invitrogen, Inc., Carlsbad, CA), and the BD BaculoGold™ (BD Biosciences-Pharmigen, San Diego, CA). Insect expression systems include, without limitation, the *Drosophila* Expression System (DES®) (Invitrogen, Inc., Carlsbad, CA), InsectSelect™ System (Invitrogen, Inc., Carlsbad, CA) and InsectDirect™ System (EMD Biosciences-Novagen, Madison, WI). Non-limiting examples of mammalian expression systems include the T-REx™ (Tetracycline-Regulated Expression) System (Invitrogen, Inc., Carlsbad, CA), the Flp-In™ T-REx™ System (Invitrogen, Inc., Carlsbad, CA), the pcDNA™ system (Invitrogen, Inc., Carlsbad, CA), the pSecTag2 system (Invitrogen, Inc., Carlsbad, CA), the Exchanger® System, InterPlay™ Mammalian TAP System (Stratagene, La Jolla, CA), Complete Control® Inducible Mammalian Expression System (Stratagene, La Jolla, CA) and LacSwitch® II Inducible Mammalian Expression System (Stratagene, La Jolla, CA).

[0397] Another procedure of expressing a modified Clostridial toxin encoded by polynucleotide molecule disclosed in the present specification employs a cell-free expression system such as, without limitation, prokaryotic extracts and eukaryotic extracts. Non-limiting examples of prokaryotic cell extracts include the RTS 100 *E. coli* HY Kit (Roche Applied Science, Indianapolis, IN), the ActivePro In Vitro Translation Kit (Ambion, Inc., Austin, TX), the EcoPro™ System (EMD Biosciences-Novagen, Madison, WI) and the Expressway™ Plus Expression System (Invitrogen, Inc., Carlsbad, CA). Eukaryotic cell extract include, without limitation, the RTS 100 Wheat Germ CECF Kit (Roche Applied Science, Indianapolis, IN), the TnT® Coupled Wheat Germ Extract Systems (Promega Corp., Madison, WI), the Wheat Germ IVT™ Kit (Ambion, Inc., Austin, TX), the Retic Lysate IVT™ Kit (Ambion, Inc., Austin, TX), the PROTEINscript® II System (Ambion, Inc., Austin, TX) and the TnT® Coupled Reticulocyte Lysate Systems (Promega Corp., Madison, WI).

[0398] Other aspects of this invention can be described as follows:

1. A modified Clostridial toxin comprising:

    a) a Clostridial toxin enzymatic domain capable of executing an enzymatic target modification step of a Clostridial toxin intoxication process;
    b) a Clostridial toxin translocation domain capable of executing a translocation step of a Clostridial toxin intoxication process;
    c) a translocation facilitating domain capable of facilitating a translocation step of a Clostridial toxin intoxication process;
    d) an altered targeting domain capable of selectively binding a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell and executing a cell binding step of a Clostridial toxin intoxication process; and
    e) a protease cleavage site

    wherein cleavage of the protease cleavage site converts the single-chain form of the modified Clostridial toxin into the di-chain form.

2. The modified Clostridial toxin according to 1, wherein the modified Clostridial toxin comprises, in a linear amino-to-carboxyl single polypeptide order, the Clostridial toxin enzymatic domain, the protease cleavage site, the Clostridial toxin translocation domain, the translocation facilitating domain and the altered targeting domain.

3. The modified Clostridial toxin according to 1, wherein the modified Clostridial toxin comprises, in a linear amino-to-carboxyl single polypeptide order, the Clostridial toxin enzymatic domain, the protease cleavage site, the altered targeting domain, the Clostridial toxin translocation domain and the translocation facilitating domain.

4. The modified Clostridial toxin according to 1, wherein the modified Clostridial toxin comprises, in a linear amino-to-carboxyl single polypeptide order, the altered targeting domain, the Clostridial toxin translocation domain, the translocation facilitating domain, the protease cleavage site and the Clostridial toxin enzymatic domain.

5. The modified Clostridial toxin according to 1, wherein the modified Clostridial toxin comprises, in a linear amino-to-carboxyl single polypeptide order, the altered targeting domain, the Clostridial toxin enzymatic domain, the protease cleavage site, the Clostridial toxin translocation domain and the translocation facilitating domain.

6. The modified Clostridial toxin according to 1, wherein the modified Clostridial toxin comprises, in a linear amino-to-carboxyl single polypeptide order, the Clostridial toxin translocation domain, the

translocation facilitating domain, the protease cleavage site, the Clostridial toxin enzymatic domain and the altered targeting domain.

7. The modified Clostridial toxin according to 1, wherein the modified Clostridial toxin comprises, in a linear amino-to-carboxyl single polypeptide order, the Clostridial toxin translocation domain, the translocation facilitating domain, the protease cleavage site, the altered targeting domain and the Clostridial toxin enzymatic domain.

8. The modified Clostridial toxin according to 1, wherein the Clostridial toxin enzymatic domain is selected from the group consisting of a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain and a TeNT enzymatic domain.

9. The modified Clostridial toxin according to 1, wherein the Clostridial toxin translocation domain is selected from the group consisting of a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain and a TeNT translocation domain.

10. The modified Clostridial toxin according to 1, wherein the translocation facilitating domain is a Clostridial toxin translocation facilitating domain.

11. The modified Clostridial toxin according to 10, wherein the Clostridial toxin translocation facilitating domain is selected from the group consisting of a BoNT/A translocation facilitating domain, a BoNT/B translocation facilitating domain, a BoNT/C1 translocation facilitating domain, a BoNT/D translocation domain, a BoNT/E translocation facilitating domain, a BoNT/F translocation facilitating domain, a BoNT/G translocation facilitating domain and a TeNT translocation facilitating domain.

12. The modified Clostridial toxin according to 10, wherein the Clostridial toxin translocation facilitating domain comprises an amino acid sequence selected from the group consisting of amino acids 874-1110 of SEQ ID NO: 1, amino acids 861-1097of SEQ ID NO: 2, amino acids 869-1111 of SEQ ID NO: 3, amino acids 865-1098 of SEQ ID NO: 4, amino acids 848-1085 of SEQ ID NO: 5, amino acids 867-1105 of SEQ ID NO: 6, amino acids 866-1105 of SEQ ID NO: 7 and amino acid 882-1127 of SEQ ID NO: 8.

13. The modified Clostridial toxin according to 10, wherein the Clostridial toxin translocation facilitating domain is selected from the group consisting of a BoNT/A translocation facilitating domain, a BoNT/B translocation facilitating domain, a BoNT/C1 translocation facilitating domain, a BoNT/D translocation

domain, a BoNT/E translocation facilitating domain, a BoNT/F translocation facilitating domain, a BoNT/G translocation facilitating domain and a TeNT translocation facilitating domain.

14. The modified Clostridial toxin according to 1, wherein the translocation facilitating domain is an enveloped virus fusogenic peptide domain.

15. The modified Clostridial toxin according to 14, wherein the enveloped virus fusogenic peptide domain is selected from the group consisting of an influenzavirus fusogenic peptide domain, an alphavirus fusogenic peptide domain, a vesiculovirus fusogenic peptide domain, a respirovirus fusogenic peptide domain, a morbillivirus fusogenic peptide domain, an avulavirus fusogenic peptide domain, a henipavirus fusogenic peptide domain, a metapneumovirus fusogenic peptide domain and a foamy virus fusogenic peptide domain.

16. The modified Clostridial toxin according to 15, wherein the influenzavirus fusogenic peptide domain is selected from the group consisting of SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197 and SEQ ID NO: 198.

17. The modified Clostridial toxin according to 15, wherein the alphavirus fusogenic peptide domain is selected from the group consisting of SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224 and SEQ ID NO: 225.

18. The modified Clostridial toxin according to 15, wherein the vesiculovirus fusogenic peptide domain is selected from the group consisting of SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235 and SEQ ID NO: 236.

19. The modified Clostridial toxin according to 15, wherein the respirovirus fusogenic peptide domain is selected from the group consisting of SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242 and SEQ ID NO: 243.

20. The modified Clostridial toxin according to 15, wherein the morbillivirus fusogenic peptide domain is selected from the group consisting of SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252 and SEQ ID NO: 253.

21. The modified Clostridial toxin according to 15, wherein the avulavirus fusogenic peptide domain is selected from the group consisting of SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265 and SEQ ID NO: 266.

22. The modified Clostridial toxin according to 15, wherein the henipavirus fusogenic peptide domain is selected from the group consisting of SEQ ID NO: 267 and SEQ ID NO: 268.

23. The modified Clostridial toxin according to 15, wherein the metapneumovirus fusogenic peptide domain is SEQ ID NO: 269.

24. The modified Clostridial toxin according to 1, wherein the altered targeting domain is selected from the group consisting of an opioid, a melanocortin, a galanin, a granin, a tachykinin, a cholecystokinin, a Neuropeptide Y related, a kinin peptide, a PAR peptide, a corticotropin-releasing hormone, a thyrotropin-releasing hormone and a somatostatin.

25. The modified Clostridial toxin according to 24, wherein the altered targeting domain comprises an opioid selected from the group consisting of an enkephalin, a BAM22 peptide, an endomorphin, an endorphin, a dynorphin, a nociceptin and a hemorphin.

26. The modified Clostridial toxin according to 25, wherein the altered targeting domain comprises an enkephalin selected from the group consisting of a Leu-enkephalin, a Met-enkephalin, a Met-enkephalin MRGL and a Met-enkephalin MRF

27. The modified Clostridial toxin according to 25, wherein the altered targeting domain comprises an enkephalin selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12.

28. The modified Clostridial toxin according to 25, wherein the altered targeting domain comprises a BAM22 peptide selected from the group consisting of a BAM22 peptide (1-12), a BAM22 peptide (6-22), a BAM22 peptide (8-22) or a BAM22 peptide (1-22).

29. The modified Clostridial toxin according to 25, wherein the altered targeting domain comprises a BAM22 peptide selected from the group consisting of amino acids 1-12 of SEQ ID NO: 172, amino acids 6-22 of SEQ ID NO: 172, amino acids 8-22 of SEQ ID NO: 172, amino acids 1-22 of SEQ ID NO: 172, amino acids 1-12 of SEQ ID NO: 173, amino acids 6-22 of SEQ ID NO: 173, amino acids 8-22 of SEQ ID NO: 173, amino acids 1-22 of SEQ ID NO: 173, amino acids 1-12 of SEQ ID NO: 174, amino acids 6-22 of SEQ ID NO: 174, amino acids 8-22 of SEQ ID NO: 174, amino acids 1-22 of SEQ ID NO: 174, amino acids 1-12 of SEQ ID NO: 175, amino acids 6-22 of SEQ ID NO: 175, amino

acids 8-22 of SEQ ID NO: 175, amino acids 1-22 of SEQ ID NO: 175, amino acids 1-12 of SEQ ID NO: 176, amino acids 6-22 of SEQ ID NO: 176, amino acids 8-22 of SEQ ID NO: 176, amino acids 1-22 of SEQ ID NO: 176, amino acids 1-12 of SEQ ID NO: 177, amino acids 6-22 of SEQ ID NO: 177, amino acids 8-22 of SEQ ID NO: 177 and amino acids 1-22 of SEQ ID NO: 177.

30. The modified Clostridial toxin according to 25, wherein the altered targeting domain comprises an endomorphin selected from the group consisting of an endomorphin-1 and an endomorphin-2.

31. The modified Clostridial toxin according to 25, wherein the altered targeting domain comprises an endomorphin selected from the group consisting of SEQ ID NO: 13 and SEQ ID NO: 14.

32. The modified Clostridial toxin according to 25, wherein the altered targeting domain comprises an endorphin selected from the group consisting of an endorphin-α, a neoendorphin-α, an endorphin-β, a neoendorphin-β or an endorphin-γ.

33. The modified Clostridial toxin according to 25, wherein the altered targeting domain comprises an endorphin selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20.

34. The modified Clostridial toxin according to 25, wherein the altered targeting domain comprises a dynorphin selected from the group consisting of a dynorphin A, a dynorphin B and a rimorphin.

35. The modified Clostridial toxin according to 25, wherein the altered targeting domain comprises a dynorphin selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50 and SEQ ID NO: 51.

36. The modified Clostridial toxin according to 25, wherein the altered targeting domain comprises a nociceptin selected from the group consisting of a nociceptin RK, a nociceptin, a neuropeptide 1, a neuropeptide 2 or a neuropeptide 3.

37. The modified Clostridial toxin according to 25, wherein the altered targeting domain comprises a nociceptin selected from the group consisting of SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60 and SEQ ID NO: 61.

38. The modified Clostridial toxin according to 24, wherein the altered targeting domain comprises a melanocortin selected from the group consisting of an α-melanocyte stimulating hormones (α-MSH), a β-melanocyte stimulating hormones (β-MSH), a γ-melanocyte stimulating hormones (γ-MSH), an adrenocorticotropin (ACTH), a Corticotropin-like intermediary peptide (CLIP), a β-lipotropin (β-LPH) and a γ-lipotropin (γ-LPH).

39. The modified Clostridial toxin according to 24, wherein the altered targeting domain comprises a melanocortin selected from the group consisting SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70 and SEQ ID NO: 71.

40. The modified Clostridial toxin according to 11, wherein the altered targeting domain comprises a galanin selected from the group consisting of a galanin and a galanin message-associated peptide (GMAP).

41. The modified Clostridial toxin according to 24, wherein the altered targeting domain comprises a galanin selected from the group consisting of SEQ ID NO: 72 and SEQ ID NO: 73.

42. The modified Clostridial toxin according to 24, wherein the altered targeting domain comprises a grainin selected from the group consisting of a chromogranin A peptide, a chromogranin B peptide and a chromogranin C peptide.

43. The modified Clostridial toxin according to 42, wherein the altered targeting domain comprises a chromogranin A peptide selected from the group consisting of a β-granin, a vasostatin, a chromostatin, a pancreastatin, a WE-14, a catestatin, a parastatin and a GE-25.

44. The modified Clostridial toxin according to 42, wherein the altered targeting domain comprises a chromogranin A peptide selected from the group consisting of SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 and SEQ ID NO: 81

45. The modified Clostridial toxin according to 42, wherein the altered targeting domain comprises a chromogranin B peptide selected from the group consisting of a GAWK peptide, an adrenomedullary peptide and a secretolytin.

46. The modified Clostridial toxin according to 42, wherein the altered targeting domain comprises a chromogranin B peptide selected from the group consisting of SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85 and SEQ ID NO: 86

47. The modified Clostridial toxin according to 42, wherein the altered targeting domain comprises a chromogranin C peptide is a secretoneurin, a EM66 and a manserin.

48. The modified Clostridial toxin according to 42, wherein the altered targeting domain comprises a chromogranin C peptide is SEQ ID NO: 87.

49. The modified Clostridial toxin according to 24, wherein the altered targeting domain comprises a tachykinin peptide selected from the group consisting of a Substance P, a neuropeptide K (NPK), a neuropeptide gamma, a neurokinin A, a neurokinin B, a hemokinin and a endokinin.

50. The modified Clostridial toxin according to 24, wherein the altered targeting domain comprises a tachykinin peptide selected from the group consisting of SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 and SEQ ID NO: 99.

51. The modified Clostridial toxin according to 24, wherein the altered targeting domain comprises a cholecystokinin peptide selected from the group consisting of a cholecystokinin 58, a cholecystokinin 39, a cholecystokinin 33, a cholecystokinin 12 and a cholecystokinin 8.

52. The modified Clostridial toxin according to 51, wherein the altered targeting domain comprises a cholecystokinin 58 peptide selected from the group consisting of SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115.

53. The modified Clostridial toxin according to 51, wherein the altered targeting domain comprises a cholecystokinin 39 peptide selected from the group consisting of amino acids 20-58 of SEQ ID NO: 100, amino acids 20-58 of SEQ ID NO: 101, amino acids 20-58 of SEQ ID NO: 102, amino acids 20-58 of SEQ ID NO: 103, amino acids 20-58 of SEQ ID NO: 104, amino acids 20-58 of SEQ ID NO: 105, amino acids 20-58 of SEQ ID NO: 107, amino acids 20-58 of SEQ ID NO: 108, amino acids 20-58 of SEQ ID NO: 109, amino acids 20-58 of SEQ ID NO: 110, amino acids 20-58 of SEQ ID NO: 111, amino acids 20-58 of SEQ ID NO: 112, SEQ ID NO: 113, amino acids 20-58 of SEQ ID NO: 114 or amino acids 20-58 of SEQ ID NO: 115.

54. The modified Clostridial toxin according to 51, wherein the altered targeting domain comprises a cholecystokinin 33 peptide selected from the group consisting of amino acids 26-58 of SEQ ID NO: 100, amino acids 26-58 of SEQ ID NO: 101, amino acids 26-58 of SEQ ID NO: 102, amino acids 26-58 of SEQ ID NO: 103, amino acids 26-58 of SEQ ID NO: 104, amino acids 26-58 of SEQ ID NO:

105, amino acids 26-58 of SEQ ID NO: 107, amino acids 26-58 of SEQ ID NO: 108, amino acids 26-58 of SEQ ID NO: 109, amino acids 26-58 of SEQ ID NO: 110, amino acids 26-58 of SEQ ID NO: 111, amino acids 26-58 of SEQ ID NO: 112, amino acids 26-58 of SEQ ID NO: 113, amino acids 26-58 of SEQ ID NO: 114 and amino acids 26-58 of SEQ ID NO: 115.

55. The modified Clostridial toxin according to 51, wherein the altered targeting domain comprises a cholecystokinin 12 peptide selected from the group consisting of amino acids 47-58 of SEQ ID NO: 100, amino acids 47-58 of SEQ ID NO: 110 and amino acids 47-58 of SEQ ID NO: 114.

56. The modified Clostridial toxin according to 51, wherein the altered targeting domain comprises a cholecystokinin 8 peptide is amino acids 51-58 of SEQ ID NO: 100.

57. The modified Clostridial toxin according to 24, wherein the altered targeting domain comprises a Neuropeptide Y related peptide selected from the group consisting of a Neuropeptide Y (NPY), a Peptide YY (PYY), a Pancreatic peptide (PP) and a Pancreatic icosapeptide (PIP).

58. The modified Clostridial toxin according to 24, wherein the altered targeting domain comprises a Neuropeptide Y related peptide selected from the group consisting of SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 and SEQ ID NO: 120.

59. The modified Clostridial toxin according to 24, wherein the altered targeting domain comprises a kinin peptide selected from the group consisting of a bradykinin, a kallidin, a desArg^{9} bradykinin and a desArg^{10} bradykinin.

60. The modified Clostridial toxin according to 24, wherein the altered targeting domain comprises a kinin peptide selected from the group consisting of SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180 and SEQ ID NO: 181.

61. The modified Clostridial toxin according to 24, wherein the altered targeting domain comprises a PAR peptide selected from the group consisting of a PAR1 peptide, a PAR2 peptide, a PAR3 peptide and a PAR4 peptide.

62. The modified Clostridial toxin according to 24, wherein the altered targeting domain comprises a PAR peptide selected from the group consisting of SEQ ID NO: 182, SEQ ID NO: 283, SEQ ID NO: 184 and SEQ ID NO: 185. amino acids 42-47 of SEQ ID NO: 182, amino acids 42-55 of SEQ ID NO: 182, amino acids 29-64 of SEQ ID NO: 182, amino acids 1-64 of SEQ ID NO: 182, amino acids 35-40 of SEQ ID NO: 183, amino acids 35-48 of SEQ ID NO: 183, amino acids 24-59 of SEQ ID NO: 183, amino acids 1-59 of SEQ ID NO: 183, amino acids 39-44 of SEQ ID NO: 184, amino acids 39-52 of SEQ ID NO: 184, amino acids 26-60 of SEQ ID NO: 184, amino acids 1-60 of SEQ ID NO: 184,

amino acids 48-53 of SEQ ID NO: 185, amino acids 48-61 of SEQ ID NO: 185, amino acids 35-70 of SEQ ID NO: 185 and amino acids 1-70 of SEQ ID NO: 185.

63. The modified Clostridial toxin according to 24, wherein the altered targeting domain is selected from the group consisting of SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 186 and SEQ ID NO: 187.

64. The modified Clostridial toxin according to 1, wherein the protease cleavage site is an endogenous Clostridial toxin di-chain loop protease cleavage site or an exogenous cleavage site.

65. The modified Clostridial toxin according to 64, wherein the endogenous Clostridial toxin di-chain loop protease cleavage site is selected from the group consisting of a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site and a TeNT di-chain loop protease cleavage site.

66. The modified Clostridial toxin according to 64, wherein the exogenous protease cleavage site is selected from the group consisting of an enterokinase cleavage site, a Thrombin cleavage site, a Factor Xa cleavage site, a human rhinovirus 3C protease cleavage site, a tobacco etch virus protease cleavage site, a dipeptidyl aminopeptidase cleavage site, a small ubiquitin-like modifier (SUMO)/ubiquitin-like protein-1(ULP-1) protease cleavage site, and a Clostridial toxin substrate cleavage site.

67. The modified Clostridial toxin according to 66, wherein the Clostridial toxin substrate cleavage site is selected from the group consisting of a BoNT/A substrate cleavage site, a BoNT/B substrate cleavage site, a BoNT/C1 substrate cleavage site, a BoNT/D substrate cleavage site, a BoNT/E substrate cleavage site, a BoNT/F substrate cleavage site, a BoNT/G substrate cleavage site and a TeNT substrate cleavage site.

68. A polynucleotide molecule encoding a modified Clostridial toxin, the polynucleotide molecule comprising:

a) a polynucleotide molecule encoding a Clostridial toxin enzymatic domain capable of executing an enzymatic target modification step of a Clostridial toxin intoxication process;
b) a polynucleotide molecule encoding a Clostridial toxin translocation domain capable of executing a translocation step of a Clostridial toxin intoxication process;
c) a polynucleotide molecule encoding a translocation facilitating domain capable of facilitating a translocation step of a Clostridial toxin intoxication process;

d) a polynucleotide molecule encoding an altered targeting domain capable of selectively binding a non-Clostridial toxin receptor present on a Clostridial toxin target cell and executing a cell binding step of a Clostridial toxin intoxication process; and

e) a polynucleotide molecule encoding a protease cleavage site

wherein cleavage of the protease cleavage site converts the single-chain form of the modified Clostridial toxin into the di-chain form.

69. The polynucleotide molecule according to 68, wherein the polynucleotide molecule encodes the modified Clostridial toxin of any one of Claims 1-7.

70. The polynucleotide molecule according to 68, wherein the polynucleotide molecule encoding a Clostridial toxin enzymatic domain selected from the group consisting of a polynucleotide molecule encoding a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain and a TeNT enzymatic domain.

71. The polynucleotide molecule according to 68, wherein the polynucleotide molecule encoding a Clostridial toxin translocation domain selected from the group consisting of a polynucleotide molecule encoding a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain and a TeNT translocation domain.

72. The polynucleotide molecule according to 68, wherein the translocation facilitating domain is a Clostridial toxin translocation facilitating domain.

73. The polynucleotide molecule according to 72, wherein the Clostridial toxin translocation facilitating domain is selected from the group consisting of a BoNT/A translocation facilitating domain, a BoNT/B translocation facilitating domain, a BoNT/C1 translocation facilitating domain, a BoNT/D translocation domain, a BoNT/E translocation facilitating domain, a BoNT/F translocation facilitating domain, a BoNT/G translocation facilitating domain and a TeNT translocation facilitating domain.

74. The polynucleotide molecule according to 68, wherein the translocation facilitating domain is an enveloped virus fusogenic peptide domain.

75. The polynucleotide molecule according to 74, wherein the enveloped virus fusogenic peptide domain is selected from the group consisting of an influenzavirus fusogenic peptide domain, an alphavirus fusogenic peptide domain, a vesiculovirus fusogenic peptide domain, a respirovirus fusogenic peptide

domain, a morbillivirus fusogenic peptide domain, an avulavirus fusogenic peptide domain, a henipavirus fusogenic peptide domain, a metapneumovirus fusogenic peptide domain and a foamy virus fusogenic peptide domain.

76. The polynucleotide molecule according to 68, wherein the polynucleotide molecule encoding the altered targeting domain is selected from the group consisting of a polynucleotide molecule encoding an opioid, a melanocortin, a galanin, a granin, a tachykinin, a cholecystokinin, a Neuropeptide Y related, a kinin peptide, a PAR peptide, a corticotropin-releasing hormone, a thyrotropin-releasing hormone and a somatostatin.

77. The polynucleotide molecule according to 68, wherein the polynucleotide molecule encoding the altered targeting domain comprising an opioid selected from the group consisting of an enkephalin, a BAM22 peptide, an endomorphin, an endorphin, a dynorphin, a nociceptin and a hemorphin.

78. The polynucleotide molecule according to 68, wherein the polynucleotide molecule encoding the altered targeting domain comprises a melanocortin selected from the group consisting of a polynucleotide molecule encoding an α-melanocyte stimulating hormones (α-MSH), a β-melanocyte stimulating hormones (β-MSH), a γ-melanocyte stimulating hormones (γ-MSH), an adrenocorticotropin (ACTH), a Corticotropin-like intermediary peptide (CLIP), a β-lipotropin (β-LPH) and a γ-lipotropin (γ-LPH).

79. The polynucleotide molecule according to 68, wherein the polynucleotide molecule encoding the altered targeting domain comprises a galanin selected from the group consisting of a polynucleotide molecule encoding a galanin and a galanin message-associated peptide (GMAP).

80. The polynucleotide molecule according to 68, wherein the polynucleotide molecule encoding the altered targeting domain comprises a grainin selected from the group consisting of a polynucleotide molecule encoding a chromogranin A peptide, a chromogranin B peptide and a chromogranin C peptide.

81. The polynucleotide molecule according to 80, wherein the polynucleotide molecule encoding the altered targeting domain comprises a chromogranin A peptide selected from the group consisting of a polynucleotide molecule encoding a β-granin, a vasostatin, a chromostatin, a pancreastatin, a WE-14, a catestatin, a parastatin and a GE-25.

82. The polynucleotide molecule according to 80, wherein the polynucleotide molecule encoding the altered targeting domain comprises a chromogranin B peptide selected from the group consisting of a polynucleotide molecule encoding a GAWK peptide, an adrenomedullary peptide and a secretolytin.

83. The polynucleotide molecule according to 80, wherein the polynucleotide molecule encoding the altered targeting domain comprises a chromogranin C peptide is a polynucleotide molecule encoding a secretoneurin.

84. The polynucleotide molecule according to 68, wherein the polynucleotide molecule encoding the altered targeting domain comprises a tachykinin peptide selected from the group consisting of a polynucleotide molecule encoding a Substance P, a neuropeptide K (NPK), a neuropeptide gamma, a neurokinin A, a neurokinin B, a hemokinin and a endokinin.

85. The polynucleotide molecule according to 68, wherein the polynucleotide molecule encoding the altered targeting domain comprises a cholecystokinin peptide selected from the group consisting of a polynucleotide molecule encoding a cholecystokinin 58, a cholecystokinin 39, a cholecystokinin 33, a cholecystokinin 12 and a cholecystokinin 8.

86. The polynucleotide molecule according to 68, wherein the polynucleotide molecule encoding the altered targeting domain comprises a Neuropeptide Y related peptide selected from the group consisting of a polynucleotide molecule encoding a Neuropeptide Y (NPY), a Peptide YY (PYY), a Pancreatic peptide (PP) and a Pancreatic icosapeptide (PIP).

87. The polynucleotide molecule according to 68, wherein the polynucleotide molecule encoding the altered targeting domain comprises a kinin peptide selected from the group consisting of a polynucleotide molecule encoding a bradykinin, a kallidin, a $desArg^9$ bradykinin and a $desArg^{10}$ bradykinin.

88. The polynucleotide molecule according to 68, wherein the polynucleotide molecule encoding the altered targeting domain comprises a PAR peptide selected from the group consisting of a polynucleotide molecule encoding a PAR1, a PAR2, a PAR3 and a PAR4.

89. A method of producing a modified Clostridial toxin comprising the step of expressing a polynucleotide molecule encoding a modified Clostridial toxin in a cell, the polynucleotide molecule comprising:

a) a polynucleotide molecule encoding a Clostridial toxin enzymatic domain capable of executing an enzymatic target modification step of a Clostridial toxin intoxication process;
b) a polynucleotide molecule encoding a Clostridial toxin translocation domain capable of executing a translocation step of a Clostridial toxin intoxication process;
c) a polynucleotide molecule encoding a translocation facilitating domain capable of facilitating a translocation step of a Clostridial toxin intoxication process;

d) a polynucleotide molecule encoding an altered targeting domain capable of selectively binding a non-Clostridial toxin receptor present on a Clostridial toxin target cell and executing a cell binding step of a Clostridial toxin intoxication process; and

e) a polynucleotide molecule encoding a protease cleavage site

wherein cleavage of the protease cleavage site converts the single-chain form of the modified Clostridial toxin into the di-chain form.

90. The method according to 89, wherein the polynucleotide molecule is any one of the polynucleotide molecules of 69.

91. A method of producing a modified Clostridial toxin comprising the steps of:

a) introducing into a cell a polynucleotide molecule encoding a modified Clostridial toxin, the polynucleotide molecule comprising:

i) a polynucleotide molecule encoding a Clostridial toxin enzymatic domain capable of executing an enzymatic target modification step of a Clostridial toxin intoxication process;

ii) a polynucleotide molecule encoding a Clostridial toxin translocation domain capable of executing a translocation step of a Clostridial toxin intoxication process;

iii) a polynucleotide molecule encoding a translocation facilitating domain capable of facilitating a translocation step of a Clostridial toxin intoxication process;

iv) a polynucleotide molecule encoding an altered targeting domain capable of selectively binding a non-Clostridial toxin receptor present on a Clostridial toxin target cell and executing a cell binding step of a Clostridial toxin intoxication process; and

v) a polynucleotide molecule encoding a protease cleavage site

wherein cleavage of the protease cleavage site converts the single-chain form of the modified Clostridial toxin into the di-chain form.

b) expressing the modified Clostridial toxin encoded by the polynucleotide molecule.

92. The method according to 91, wherein the polynucleotide molecule is any one of the polynucleotide molecules of 69.

EXAMPLES

[0399] The following non-limiting examples are provided for illustrative purposes only in order to facilitate a more complete understanding of disclosed embodiments and are in no way intended to limit any of the embodiments disclosed in the present specification.

Example 1

Construction of a modified Clostridial toxin comprising a translocation facilitating domain and an amino-terminally presented altered targeting domain

[0400] This example illustrates how to make a modified Clostridial toxin disclosed in the present specification comprising a translocation facilitating domain and an altered targeting domain located at the amino terminus of the modified toxin.

1a. A targeting-translocation-translocation facilitating-enzymatic domain organization.

[0401] A polynucleotide molecule based on BoNT/A-AP4A-Nociceptin (SEQ ID NO: 188) will be synthesized using standard procedures (BlueHeron® Biotechnology, Bothell, WA). This polynucleotide molecule encodes a BoNT/A modified to replace amino acids 1111-1296 of SEQ ID NO: 1, a BoNT/A H_{CC} targeting domain, with SEQ ID NO: 52, a nociceptin-RK targeting domain, and has the general domain arrangement of FIG. 4A. In addition to the nociceptin-RK targeting domain, the altered targeting domain further comprises at its amino terminus, a PAR 1 leader sequence ending in an enterokinse cleavage site. Cleavage of this site results in exposing the first amino acid of the nociceptin-RK targeting domain. Oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides will be hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule will be cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-AP4A-Nociceptin. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA).

[0402] If desired, an expression optimized polynucleotide molecule based on BoNT/A-AP4A-Nociceptin (SEQ ID NO: 188) can be synthesized in order to improve expression in an *Escherichia coli* strain. The polynucleotide molecule encoding the BoNT/A-AP4A-Nociceptin will be modified to 1) contain synonymous codons typically present in native polynucleotide molecules of an *Escherichia coli* strain; 2) contain a G+C content that more closely matches the average G+C content of native polynucleotide molecules found in an *Escherichia coli* strain; 3) reduce polymononucleotide regions found within the polynucleotide molecule; and/or 4) eliminate internal regulatory or structural sites found within the polynucleotide molecule, see, *e.g.*, Lance E. Steward *et al.*, *Optimizing Expression of Active Botulinum Toxin Type E*, International Patent Publication WO 2006/011966 (Feb. 2, 2006); Lance E. Steward *et al.*, *Optimizing Expression of Active Botulinum Toxin Type A*, International Patent Publication WO

2006/017749 (Feb. 16, 2006). Once sequence optimization is complete, oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-AP4A-Nociceptin. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA). If so desired, expression optimization to a different organism, such as, *e.g.*, a yeast strain, an insect cell-line or a mammalian cell line, can be done, see, *e.g.*, Steward, *supra*, (Feb. 2, 2006); and Steward, *supra*, (Feb. 16, 2006).

[0403] A similar cloning strategy will be used to make pUCBHB1 cloning constructs for BoNT/B-AP4A-Nociceptin, a modified BoNT/B where amino acids 1098-1291 of SEQ ID NO: 2 are replaced with SEQ ID NO: 52; BoNT/C1-AP4A-Nociceptin, a modified BoNT/C1 where amino acids 1112-1291 of SEQ ID NO: 3 are replaced with SEQ ID NO: 52; BoNT/D-AP4A-Nociceptin, a modified BoNT/D where amino acids 1099-1276 of SEQ ID NO: 4 are replaced with SEQ ID NO: 52; BoNT/E-AP4A-Nociceptin, a modified BoNT/E where amino acids 1086-1252 of SEQ ID NO: 5 are replaced with SEQ ID NO: 52; BoNT/F-AP4A-Nociceptin, a modified BoNT/F where amino acids 1106-1274 of SEQ ID NO: 6 are replaced with SEQ ID NO: 52; BoNT/G-AP4A-Nociceptin, a modified BoNT/G where amino acids 1106-1297 of SEQ ID NO: 7 are replaced with SEQ ID NO: 52; and TeNT-AP4A-Nociceptin, a modified TeNT where amino acids 1128-1315 of SEQ ID NO: 8 are replaced with SEQ ID NO: 52.

[0404] Likewise, a similar cloning strategy will be used to make pUCBHB1 cloning constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-AP4A that will replace the H_{CC} targeting domain from a Clostridial toxin the with an altered targeting domain comprising, *e.g*, altered targeting domains SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184; and altered targeting domains requiring a free amino-terminal amino acid like SEQ ID NO: 9 to SEQ ID NO: 51 or SEQ ID NO: 53 to SEQ ID NO: 61, as well as, amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177; amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; or amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

[0405] To construct pET29/BoNT/A-AP4A-Nociceptin, a pUCBHB1/BoNT/A-AP4A-Nociceptin construct will be digested with restriction endonucleases that 1) will excise the polynucleotide molecule encoding the open reading frame of BoNT/A-AP4A-Nociceptin; and 2) will enable this polynucleotide molecule to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). This insert will be subcloned using a T4 DNA ligase procedure into a pET29 vector that is digested with appropriate restriction endonucleases to yield pET29/BoNT/A-AP4A-Nociceptin. The ligation mixture will be transformed into chemically competent *E. coli* DH5α cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, will be plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 50 μg/mL of Kanamycin, and will be placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs will be identified as Kanamycin resistant colonies. Candidate constructs will be isolated using an alkaline lysis plasmid mini-preparation procedure and will be analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy will yield a pET29 expression construct comprising the polynucleotide molecule encoding the BoNT/A-AP4A-Nociceptin operably-linked to a carboxyl terminal polyhistidine affinity binding peptide.

[0406] A similar cloning strategy will be used to make pET29 expression constructs for other modified Clostridial toxin-AP4A-Nociceptin toxins, such as, *e.g.*, BoNT/B-AP4A-Nociceptin, BoNT/C1-AP4A-Nociceptin, BoNT/D-AP4A-Nociceptin, BoNT/E-AP4A-Nociceptin, BoNT/F-AP4A-Nociceptin, BoNT/G-AP4A-Nociceptin or TeNT-AP4A-Nociceptin. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-AP4A comprising an altered targeting domain such as, *e.g*, altered targeting domains SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184; and altered targeting domains requiring a free amino-terminal amino acid like SEQ ID NO: 9 to SEQ ID NO: 51 or SEQ ID NO: 53 to SEQ ID NO: 61, as well as, amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177; amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; or amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

[0407] To construct a BoNT/A-AP4A-Nociceptin that will replace the BoNT/A translocation facilitating domain with another Clostridial toxin translocation facilitating domain, a translocation facilitating domain of BoNT/B will be introduced into the BoNT/A-AP4A-Nociceptin as described above using a Splicing by Overlapping ends polymerase chain reaction (SOE-PCR) procedure, see, *e.g.*, R. M. Horton et al., *Engineering hybrid genes without the use of restriction enzymes: gene splicing by overlapping extension*,

77(1) Gene 61-68 (1989); and R. M. Horton, *PCR-mediated recombination and mutagenesis. SOEing together tailor-made genes*, 3(2) Mol. Biotechnol. 93-99 (1995). A nucleic acid fragment comprising a region encoding amino acids 859 to 1097 of BoNT/B (SEQ ID NO: 2) will be operably-linked by SOE-PCR to replace the region corresponding to the BoNT/A translocation facilitating domain comprising amino acids 874-1110 of SEQ ID NO: 1 of the BoNT/A-AP4A-Nociceptin and will be subcloned into a pCR2.1 vector using the TOPO® TA cloning method (Invitrogen, Inc, Carlsbad, CA). The forward and reverse oligonucleotide primers used for these reactions are designed to include unique restriction enzyme sites useful for subsequent subcloning steps. The resulting construct will be digested with restriction enzymes that 1) will excise the polynucleotide molecule containing the entire open reading frame encoding the modified BoNT/A-AP4A-Nociceptin; and 2) will enable this polynucleotide molecule to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). The resulting restriction fragment will be purified by the QIAquick Gel Extraction Kit (QIAGEN, Inc., Valencia, CA), and will be subcloned using a T4 DNA ligase procedure into a pET29 vector. This cloning strategy yielded a pET29 expression construct encoding a BoNT/A-AP4A-Nociceptin comprising a BoNT/B translocation facilitating domain.

[0408] A similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a BoNT/A-AP4A-Nociceptin that replaces the region corresponding to the BoNT/A translocation facilitating domain comprising amino acids 874-1110 of SEQ ID NO: 1 with, *e.g.*, a translocation facilitating domain comprising amino acids 869-1111 of BoNT/C1 of SEQ ID NO: 3; a translocation facilitating domain comprising amino acids 865-1098 of BoNT/D of SEQ ID NO: 4; a translocation facilitating domain comprising amino acids 846-1058 of BoNT/E of SEQ ID NO: 5; a translocation facilitating domain comprising amino acids 867-1105 of BoNT/F of SEQ ID NO: 6; a translocation facilitating domain comprising amino acids 866-1105 of BoNT/G of SEQ ID NO: 7; or a translocation facilitating domain comprising amino acids 882-1127 of TeNT of SEQ ID NO: 8. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a BoNT/A-AP4A-Nociceptin that replaces the region corresponding to the BoNT/A translocation facilitating domain comprising amino acids 874-1110 of SEQ ID NO: 1 with a translocation facilitating domain comprising an enveloped virus fusogenic peptide domain, such as, *e,g,* SEQ ID NO: 194 to SEQ ID NO: 269.

[0409] Likewise, a polynucleotide molecule encoding a Clostridial translocation facilitating domain as described above can be introduced into a polynucleotide molecule encoding BoNT/B-AP4A-Nociceptin, BoNT/C1-AP4A-Nociceptin, BoNT/D-AP4A-Nociceptin, BoNT/E-AP4A-Nociceptin, BoNT/F-AP4A-Nociceptin, BoNT/G-AP4A-Nociceptin, TeNT-AP4A-Nociceptin, as well as the modified Clostridial toxin-AP4A indicated above comprising SEQ ID NO: 9 to SEQ ID NO: 51, SEQ ID NO: 53 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184, as well as, amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of

SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177; amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; or amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

1b. A targeting-enzymatic-translocation-translocation facilitating domain organization.

[0410] A polynucleotide molecule based on BoNT/A-AP4B-Nociceptin (SEQ ID NO: 189) will be synthesized and cloned into a pUCBHB1 vector as described in Example 1a. This polynucleotide molecule encodes a BoNT/A modified to replace amino acids 1111-1296 of SEQ ID NO: 1, a BoNT/A H_{CC} targeting domain, with SEQ ID NO: 52, a nociceptin-RK targeting domain, and has the general domain arrangement of FIG. 4B. In addition to the nociceptin-RK targeting domain, the altered targeting domain further comprises at its amino terminus, a PAR 1 leader sequence ending in an enterokinse cleavage site. Cleavage of this site results in exposing the first amino acid of the nociceptin-RK targeting domain. If so desired, expression optimization to a different organism, such as, *e.g.*, a bacteria, a yeast strain, an insect cell-line or a mammalian cell line, can be done as described above, see, *e.g.*, Steward, *supra*, (Feb. 2, 2006); and Steward, *supra*, (Feb. 16, 2006).

[0411] Likewise, a similar cloning strategy will be used to make pUCBHB1 cloning constructs comprising a polynucleotide molecule encoding a modified BoNT/A-AP4B with an altered targeting domain such as, *e.g*, altered targeting domains SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184; and altered targeting domains requiring a free amino-terminal amino acid like SEQ ID NO: 9 to SEQ ID NO: 51 or SEQ ID NO: 53 to SEQ ID NO: 61, as well as, amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177; amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; or amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185. In addition, similar cloning strategy will be used to produce a modified Clostridial toxin-AP4B, such as, *e.g.*, BoNT/B-AP4B, BoNT/C1-AP4B, BoNT/D-AP4B, BoNT/E-AP4B, BoNT/F-AP4B, BoNT/G-AP4B or TeNT-AP4B, to comprise an altered targeting domain comprising any one of SEQ ID NO: 9 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184, or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino

acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177; amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; or amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

[0412] To construct pET29/BoNT/A-AP4B-Nociceptin, a similar cloning strategy will be used as described in Example 1a. This cloning strategy will yield a pET29 expression construct comprising the polynucleotide molecule encoding the BoNT/A-AP4B-Nociceptin operably-linked to a carboxyl terminal polyhistidine affinity binding peptide. A similar cloning strategy will be used to make pET29 expression constructs for other modified Clostridial toxin-AP4B-Nociceptin toxins, such as, *e.g.*, BoNT/B-AP4B-Nociceptin, BoNT/C1-AP4B-Nociceptin, BoNT/D-AP4B-Nociceptin, BoNT/E-AP4B-Nociceptin, BoNT/F-AP4B-Nociceptin, BoNT/G-AP4B-Nociceptin or TeNT-AP4B-Nociceptin. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-AP4B with an altered targeting domain such as, *e.g*, altered targeting domains SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184; and altered targeting domains requiring a free amino-terminal amino acid like SEQ ID NO: 9 to SEQ ID NO: 51 or SEQ ID NO: 53 to SEQ ID NO: 61, as well as, amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177; amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; or amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

[0413] To construct a BoNT/A-AP4B-Nociceptin that will replace the BoNT/A translocation facilitating domain with another Clostridial toxin translocation facilitating domain, a similar cloning strategy using SOE-PCR will be used as described in Example 1a. This cloning strategy yielded a pET29 expression construct encoding a BoNT/A-AP4B-Nociceptin comprising a BoNT/B translocation facilitating domain, a BoNT/C1 translocation facilitating domain, a BoNT/D translocation facilitating domain, a BoNT/E translocation facilitating domain, a BoNT/F translocation facilitating domain, a BoNT/G translocation facilitating domain and a TeNT translocation facilitating domain, as well as, a translocation facilitating

domain comprising an enveloped virus fusogenic peptide domain, such as, *e,g,* SEQ ID NO: 194 to SEQ ID NO: 269.

[0414] Likewise, a polynucleotide molecule encoding a Clostridial translocation facilitating domain as described above can be introduced into a polynucleotide molecule encoding BoNT/B-AP4B-Nociceptin, BoNT/C1-AP4B-Nociceptin, BoNT/D-AP4B-Nociceptin, BoNT/E-AP4B-Nociceptin, BoNT/F-AP4B-Nociceptin, BoNT/G-AP4B-Nociceptin, TeNT-AP4B-Nociceptin, as well as the modified Clostridial toxin-AP4B indicated above comprising SEQ ID NO: 9 to SEQ ID NO: 51 or SEQ ID NO: 53 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184, as well as, amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177; amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; or amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

Example 2

Construction of a modified Clostridial toxin comprising a translocation facilitating domain and a centrally presented altered targeting domain

[0415] This example illustrates how to make a modified Clostridial toxin disclosed in the present specification comprising a translocation facilitating domain and an altered targeting domain located between two other domains of the modified toxin.

2a. An enzymatic-targeting-translocation-translocation facilitating domain organization.

[0416] A polynucleotide molecule based on BoNT/A-CP5A-Nociceptin (SEQ ID NO: 190) will be synthesized using standard procedures (BlueHeron® Biotechnology, Bothell, WA). This polynucleotide molecule encodes a BoNT/A modified to replace amino acids 1111-1296 of SEQ ID NO: 1, a BoNT/A H_{CC} targeting domain, with SEQ ID NO: 52, a nociceptin-RK targeting domain, and has the general domain arrangement of FIG. 5A. Cleavage of an enterokinse cleavage site used to form the di-chain toxin also exposes the first amino acid of the nociceptin-RK targeting domain. Oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides will be hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule will be cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-CP5A-Nociceptin. The

synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA).

[0417] If desired, an expression optimized polynucleotide molecule based on BoNT/A-CP5A-Nociceptin (SEQ ID NO: 190) can be synthesized in order to improve expression in an *Escherichia coli* strain. The polynucleotide molecule encoding the BoNT/A-CP5A-Nociceptin will be modified to 1) contain synonymous codons typically present in native polynucleotide molecules of an *Escherichia coli* strain; 2) contain a G+C content that more closely matches the average G+C content of native polynucleotide molecules found in an *Escherichia coli* strain; 3) reduce polymononucleotide regions found within the polynucleotide molecule; and/or 4) eliminate internal regulatory or structural sites found within the polynucleotide molecule, see, *e.g.*, Lance E. Steward *et al.*, *Optimizing Expression of Active Botulinum Toxin Type E*, International Patent Publication WO 2006/011966 (Feb. 2, 2006); Lance E. Steward *et al.*, *Optimizing Expression of Active Botulinum Toxin Type A*, International Patent Publication WO 2006/017749 (Feb. 16, 2006). Once sequence optimization is complete, oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-CP5A-Nociceptin. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (CP5Aplied Biosystems, Foster City, CA) and an ABI 3100 sequencer (CP5Aplied Biosystems, Foster City, CA). If so desired, expression optimization to a different organism, such as, *e.g.*, a yeast strain, an insect cell-line or a mammalian cell line, can be done, see, *e.g.*, Steward, *supra*, (Feb. 2, 2006); and Steward, *supra*, (Feb. 16, 2006).

[0418] A similar cloning strategy will be used to make pUCBHB1 cloning constructs for BoNT/B-CP5A-Nociceptin, a modified BoNT/B where amino acids 1098-1291 of SEQ ID NO: 2 are replaced with SEQ ID NO: 52; BoNT/C1-CP5A-Nociceptin, a modified BoNT/C1 where amino acids 1112-1291 of SEQ ID NO: 3 are replaced with SEQ ID NO: 52; BoNT/D-CP5A-Nociceptin, a modified BoNT/D where amino acids 1099-1276 of SEQ ID NO: 4 are replaced with SEQ ID NO: 52; BoNT/E-CP5A-Nociceptin, a modified BoNT/E where amino acids 1086-1252 of SEQ ID NO: 5 are replaced with SEQ ID NO: 52; BoNT/F-CP5A-Nociceptin, a modified BoNT/F where amino acids 1106-1274 of SEQ ID NO: 6 are replaced with SEQ ID NO: 52; BoNT/G-CP5A-Nociceptin, a modified BoNT/G where amino acids 1106-1297 of SEQ ID NO: 7 are replaced with SEQ ID NO: 52; and TeNT-CP5A-Nociceptin, a modified TeNT where amino acids 1128-1315 of SEQ ID NO: 8 are replaced with SEQ ID NO: 52.

[0419] Likewise, a similar cloning strategy will be used to make pUCBHB1 cloning constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-CP5A with an altered targeting domain such as, *e.g*, altered targeting domains SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184; and altered targeting domains requiring a free amino-terminal amino acid like SEQ ID NO: 9 to

SEQ ID NO: 51 or SEQ ID NO: 53 to SEQ ID NO: 61, as well as, amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177; amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; or amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

[0420] To construct pET29/BoNT/A-CP5A-Nociceptin, a pUCBHB1/BoNT/A-CP5A-Nociceptin construct will be digested with restriction endonucleases that 1) will excise the polynucleotide molecule encoding the open reading frame of BoNT/A-CP5A-Nociceptin; and 2) will enable this polynucleotide molecule to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). This insert will be subcloned using a T4 DNA ligase procedure into a pET29 vector that is digested with appropriate restriction endonucleases to yield pET29/BoNT/A-CP5A-Nociceptin. The ligation mixture will be transformed into chemically competent *E. coli* DH5α cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, will be plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 50 µg/mL of Kanamycin, and will be placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs will be identified as Kanamycin resistant colonies. Candidate constructs will be isolated using an alkaline lysis plasmid mini-preparation procedure and will be analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy will yield a pET29 expression construct comprising the polynucleotide molecule encoding the BoNT/A-CP5A-Nociceptin operably-linked to a carboxyl terminal polyhistidine affinity binding peptide.

[0421] A similar cloning strategy will be used to make pET29 expression constructs for other modified Clostridial toxin-CP5A-Nociceptin toxins, such as, *e.g.*, BoNT/B-CP5A-Nociceptin, BoNT/C1-CP5A-Nociceptin, BoNT/D-CP5A-Nociceptin, BoNT/E-CP5A-Nociceptin, BoNT/F-CP5A-Nociceptin, BoNT/G-CP5A-Nociceptin or TeNT-CP5A-Nociceptin. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-CP5A with an altered targeting domain such as, *e.g*, altered targeting domains SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184; and altered targeting domains requiring a free amino-terminal amino acid like SEQ ID NO: 9 to SEQ ID NO: 51 or SEQ ID NO: 53 to SEQ ID NO: 61, as well as, amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-

22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177; amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; or amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

[0422] To construct a BoNT/A-CP5A-Nociceptin that will replace the BoNT/A translocation facilitating domain with another Clostridial toxin translocation facilitating domain, a translocation facilitating domain of BoNT/B will be introduced into the BoNT/A-CP5A-Nociceptin as described above using a Splicing by Overlapping ends polymerase chain reaction (SOE-PCR) procedure, see, *e.g.*, R. M. Horton et al., *Engineering hybrid genes without the use of restriction enzymes: gene splicing by overlapping extension*, 77(1) Gene 61-68 (1989); and R. M. Horton, *PCR-mediated recombination and mutagenesis. SOEing together tailor-made genes*, 3(2) Mol. Biotechnol. 93-99 (1995). A nucleic acid fragment comprising a region encoding amino acids 859 to 1097 of BoNT/B (SEQ ID NO: 2) will be operably-linked by SOE-PCR to replace the region corresponding to the BoNT/A translocation facilitating domain comprising amino acids 874-1110 of SEQ ID NO: 1 of the BoNT/A-CP5A-Nociceptin and will be subcloned into a pCR2.1 vector using the TOPO® TA cloning method (Invitrogen, Inc, Carlsbad, CA). The forward and reverse oligonucleotide primers used for these reactions are designed to include unique restriction enzyme sites useful for subsequent subcloning steps. The resulting construct will be digested with restriction enzymes that 1) will excise the polynucleotide molecule containing the entire open reading frame encoding the modified BoNT/A-CP5A-Nociceptin; and 2) will enable this polynucleotide molecule to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). The resulting restriction fragment will be purified by the QIAquick Gel Extraction Kit (QIAGEN, Inc., Valencia, CA), and will be subcloned using a T4 DNA ligase procedure into a pET29 vector. This cloning strategy yielded a pET29 expression construct encoding a BoNT/A-CP5A-Nociceptin comprising a BoNT/B translocation facilitating domain.

[0423] A similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a BoNT/A-CP5A-Nociceptin that replaces the region corresponding to the BoNT/A translocation facilitating domain comprising amino acids 874-1110 of SEQ ID NO: 1 with, *e.g.*, a translocation facilitating domain comprising amino acids 869-1111 of BoNT/C1 of SEQ ID NO: 3; a translocation facilitating domain comprising amino acids 865-1098 of BoNT/D of SEQ ID NO: 4; a translocation facilitating domain comprising amino acids 846-1058 of BoNT/E of SEQ ID NO: 5; a translocation facilitating domain comprising amino acids 867-1105 of BoNT/F of SEQ ID NO: 6; a translocation facilitating domain comprising amino acids 866-1105 of BoNT/G of SEQ ID NO: 7; or a translocation facilitating domain comprising amino acids 882-1127 of TeNT of SEQ ID NO: 8. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a BoNT/A-CP5A-Nociceptin that replaces the region corresponding to the BoNT/A translocation facilitating domain comprising amino acids 874-1110 of SEQ ID NO: 1 with a translocation

facilitating domain comprising an enveloped virus fusogenic peptide domain, such as, *e,g,* SEQ ID NO: 194 to SEQ ID NO: 269.

[0424] Likewise, a polynucleotide molecule encoding a Clostridial translocation facilitating domain as described above can be introduced into a polynucleotide molecule encoding BoNT/B-CP5A-Nociceptin, BoNT/C1-CP5A-Nociceptin, BoNT/D-CP5A-Nociceptin, BoNT/E-CP5A-Nociceptin, BoNT/F-CP5A-Nociceptin, BoNT/G-CP5A-Nociceptin, TeNT-CP5A-Nociceptin, as well as the modified Clostridial toxin-CP5A indicated above comprising SEQ ID NO: 9 to SEQ ID NO: 51 or SEQ ID NO: 53 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184, as well as, amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177; amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; or amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

2b. A translocation-translocation facilitating-targeting-enzymatic domain organization.

[0425] A polynucleotide molecule based on BoNT/A-CP5B-Nociceptin (SEQ ID NO: 191) will be synthesized and cloned into a pUCBHB1 vector as described in Example 1a. This polynucleotide molecule encodes a BoNT/A modified to replace amino acids 1111-1296 of SEQ ID NO: 1, a BoNT/A H_{CC} targeting domain, with SEQ ID NO: 52, a nociceptin-RK targeting domain, and has the general domain arrangement of FIG. 5B. Cleavage of an enterokinse cleavage site used to form the di-chain toxin also exposes the first amino acid of the nociceptin-RK targeting domain. If so desired, expression optimization to a different organism, such as, *e.g.*, a bacteria, a yeast strain, an insect cell-line or a mammalian cell line, can be done as described above, see, *e.g.*, Steward, *supra*, (Feb. 2, 2006); and Steward, *supra*, (Feb. 16, 2006).

[0426] Likewise, a similar cloning strategy will be used to make pUCBHB1 cloning constructs comprising a polynucleotide molecule encoding a modified BoNT/A-CP5B with an altered targeting domain such as, *e.g*, altered targeting domains SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184; and altered targeting domains requiring a free amino-terminal amino acid like SEQ ID NO: 9 to SEQ ID NO: 51 or SEQ ID NO: 53 to SEQ ID NO: 61, as well as, amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino

acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177; amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 179; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 180; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 181; or amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 182.. In addition, similar cloning strategy will be used to produce a modified Clostridial toxin-CP5B, such as, *e.g.*, BoNT/B-CP5B, BoNT/C1-CP5B, BoNT/D-CP5B, BoNT/E-CP5B, BoNT/F-CP5B, BoNT/G-CP5B or TeNT-CP5B, to comprise an altered targeting domain comprising any one of SEQ ID NO: 9 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184, or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177; amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; or amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

[0427] To construct pET29/BoNT/A-CP5B-Nociceptin, a similar cloning strategy will be used as described in Example 1a. This cloning strategy will yield a pET29 expression construct comprising the polynucleotide molecule encoding the BoNT/A-CP5B-Nociceptin operably-linked to a carboxyl terminal polyhistidine affinity binding peptide. A similar cloning strategy will be used to make pET29 expression constructs for other modified Clostridial toxin-CP5B-Nociceptin toxins, such as, *e.g.*, BoNT/B-CP5B-Nociceptin, BoNT/C1-CP5B-Nociceptin, BoNT/D-CP5B-Nociceptin, BoNT/E-CP5B-Nociceptin, BoNT/F-CP5B-Nociceptin, BoNT/G-CP5B-Nociceptin or TeNT-CP5B-Nociceptin. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-CP5B with an altered targeting domain such as, *e.g*, altered targeting domains SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184; and altered targeting domains requiring a free amino-terminal amino acid like SEQ ID NO: 9 to SEQ ID NO: 51 or SEQ ID NO: 53 to SEQ ID NO: 61, as well as, amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177; amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino

acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; or amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

[0428] To construct a BoNT/A-CP5B-Nociceptin that will replace the BoNT/A translocation facilitating domain with another Clostridial toxin translocation facilitating domain, a similar cloning strategy using SOE-PCR will be used as described in Example 1a. This cloning strategy yielded a pET29 expression construct encoding a BoNT/A-CP5B-Nociceptin comprising a BoNT/B translocation facilitating domain, a BoNT/C1 translocation facilitating domain, a BoNT/D translocation facilitating domain, a BoNT/E translocation facilitating domain, a BoNT/F translocation facilitating domain, a BoNT/G translocation facilitating domain and a TeNT translocation facilitating domain, as well as, a translocation facilitating domain comprising an enveloped virus fusogenic peptide domain, such as, *e,g,* SEQ ID NO: 194 to SEQ ID NO: 269.

[0429] Likewise, a polynucleotide molecule encoding a Clostridial translocation facilitating domain as described above can be introduced into a polynucleotide molecule encoding BoNT/B-CP5B-Nociceptin, BoNT/C1-CP5B-Nociceptin, BoNT/D-CP5B-Nociceptin, BoNT/E-CP5B-Nociceptin, BoNT/F-CP5B-Nociceptin, BoNT/G-CP5B-Nociceptin, TeNT-CP5B-Nociceptin, as well as the modified Clostridial toxin-CP5B indicated above comprising SEQ ID NO: 9 to SEQ ID NO: 51 or SEQ ID NO: 53 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184, as well as, amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 172; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 173; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 174; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 175; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 176 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 177; amino acids 42-47, amino acids 42-55, amino acids 29-64 or amino acids 1-64 of SEQ ID NO: 182; amino acids 35-40, amino acids 35-48, amino acids 24-59 or amino acids 1-59 of SEQ ID NO: 183; amino acids 39-44, amino acids 39-52, amino acids 26-60 or amino acids 1-60 of SEQ ID NO: 184; or amino acids 48-53, amino acids 48-61, amino acids 35-70 or amino acids 1-70 of SEQ ID NO: 185.

Example 3

Construction of a modified Clostridial toxin comprising a translocation facilitating domain and a carboxyl-terminally presented altered targeting domain

[0430] This example illustrates how to make a modified Clostridial toxin disclosed in the present specification comprising a translocation facilitating domain and an altered targeting domain located at the carboxyl terminus of the modified toxin.

3a. An enzymatic-translocation-translocation facilitating-targeting domain organization.

[0431] A polynucleotide molecule based on BoNT/A-XP6A-Galanin (SEQ ID NO: 192) will be synthesized using standard procedures (BlueHeron® Biotechnology, Bothell, WA). This polynucleotide molecule encodes a BoNT/A modified to replace amino acids 1111-1296 of SEQ ID NO: 1, a BoNT/A H_{CC} targeting domain, with SEQ ID NO: 72, a Galanin targeting domain, and has the general domain arrangement of FIG. 6A. Oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides will be hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule will be cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-XP6A-Galanin. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA).

[0432] If desired, an expression optimized polynucleotide molecule based on BoNT/A-XP6A-Galanin (SEQ ID NO: 192) can be synthesized in order to improve expression in an *Escherichia coli* strain. The polynucleotide molecule encoding the BoNT/A-XP6A-Galanin will be modified to 1) contain synonymous codons typically present in native polynucleotide molecules of an *Escherichia coli* strain; 2) contain a G+C content that more closely matches the average G+C content of native polynucleotide molecules found in an *Escherichia coli* strain; 3) reduce polymononucleotide regions found within the polynucleotide molecule; and/or 4) eliminate internal regulatory or structural sites found within the polynucleotide molecule, see, *e.g.*, Lance E. Steward *et al.*, *Optimizing Expression of Active Botulinum Toxin Type E*, International Patent Publication WO 2006/011966 (Feb. 2, 2006); Lance E. Steward *et al.*, *Optimizing Expression of Active Botulinum Toxin Type A*, International Patent Publication WO 2006/017749 (Feb. 16, 2006). Once sequence optimization is complete, oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-XP6A-Galanin. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA). If so desired, expression optimization to a different organism, such as, *e.g.*, a yeast strain, an insect cell-line or a mammalian cell line, can be done, see, *e.g.*, Steward, *supra*, (Feb. 2, 2006); and Steward, *supra*, (Feb. 16, 2006).

[0433] A similar cloning strategy will be used to make pUCBHB1 cloning constructs for BoNT/B-XP6A-Galanin, a modified BoNT/B where amino acids 1098-1291 of SEQ ID NO: 2 are replaced with SEQ ID NO: 72; BoNT/C1-XP6A-Galanin, a modified BoNT/C1 where amino acids 1112-1291 of SEQ ID NO: 3 are replaced with SEQ ID NO: 72; BoNT/D-XP6A-Galanin, a modified BoNT/D where amino acids 1099-1276 of SEQ ID NO: 4 are replaced with SEQ ID NO: 72; BoNT/E-XP6A-Galanin, a modified BoNT/E where amino acids 1086-1252 of SEQ ID NO: 5 are replaced with SEQ ID NO: 72; BoNT/F-XP6A-Galanin, a modified BoNT/F where amino acids 1106-1274 of SEQ ID NO: 6 are replaced with SEQ ID

NO: 72; BoNT/G-XP6A-Galanin, a modified BoNT/G where amino acids 1106-1297 of SEQ ID NO: 7 are replaced with SEQ ID NO: 72; and TeNT-XP6A-Galanin, a modified TeNT where amino acids 1128-1315 of SEQ ID NO: 8 are replaced with SEQ ID NO: 72. Likewise, a similar cloning strategy will be used to make pUCBHB1 cloning constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-XP6A with an altered targeting domain such as, *e.g*, altered targeting domains SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184; and altered targeting domains requiring a free carboxyl-terminal amino acid like SEQ ID NO: 88 to SEQ ID NO: 115 and SEQ ID NO: 178 to SEQ ID NO: 181 and SEQ ID NO: 178 to SEQ ID NO: 181, as well as, amino acids 26-58 of SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115; amino acids 47-58 of SEQ ID NO: 100, SEQ ID NO: 110 or SEQ ID NO: 114; or amino acids 51-58 of SEQ ID NO: 100.

[0434] To construct pET29/BoNT/A-XP6A-Galanin, a pUCBHB1/BoNT/A-XP6A-Galanin construct will be digested with restriction endonucleases that 1) will excise the polynucleotide molecule encoding the open reading frame of BoNT/A-XP6A-Galanin; and 2) will enable this polynucleotide molecule to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). This insert will be subcloned using a T4 DNA ligase procedure into a pET29 vector that is digested with appropriate restriction endonucleases to yield pET29/BoNT/A-XP6A-Galanin. The ligation mixture will be transformed into chemically competent *E. coli* DH5α cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, will be plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 50 µg/mL of Kanamycin, and will be placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs will be identified as Kanamycin resistant colonies. Candidate constructs will be isolated using an alkaline lysis plasmid mini-preparation procedure and will be analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy will yield a pET29 expression construct comprising the polynucleotide molecule encoding the BoNT/A-XP6A-Galanin operably-linked to a carboxyl terminal polyhistidine affinity binding peptide.

[0435] A similar cloning strategy will be used to make pET29 expression constructs for other modified Clostridial toxin-XP6A-Galanin toxins, such as, *e.g.*, BoNT/B-XP6A-Galanin, BoNT/C1-XP6A-Galanin, BoNT/D-XP6A-Galanin, BoNT/E-XP6A-Galanin, BoNT/F-XP6A-Galanin, BoNT/G-XP6A-Galanin or TeNT-XP6A-Galanin. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-XP6A with an altered targeting domain such as, *e.g*, altered targeting domains SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184; and altered targeting domains requiring a free amino-terminal amino acid like SEQ ID NO: 88 to SEQ ID NO: 115 and SEQ ID NO: 178 to SEQ ID NO: 181, as well as, amino acids 26-58 of SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID

NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115; amino acids 47-58 of SEQ ID NO: 100, SEQ ID NO: 110 or SEQ ID NO: 114; or amino acids 51-58 of SEQ ID NO: 100.

[0436] To construct a BoNT/A-XP6A-Galanin that will replace the BoNT/A translocation facilitating domain with another Clostridial toxin translocation facilitating domain, a translocation facilitating domain of BoNT/B will be introduced into the BoNT/A-XP6A-Galanin as described above using a Splicing by Overlapping ends polymerase chain reaction (SOE-PCR) procedure, see, *e.g.*, R. M. Horton et al., *Engineering hybrid genes without the use of restriction enzymes: gene splicing by overlapping extension*, 77(1) Gene 61-68 (1989); and R. M. Horton, *PCR-mediated recombination and mutagenesis. SOEing together tailor-made genes*, 3(2) Mol. Biotechnol. 93-99 (1995). A nucleic acid fragment comprising a region encoding amino acids 859 to 1097 of BoNT/B (SEQ ID NO: 2) will be operably-linked by SOE-PCR to replace the region corresponding to the BoNT/A translocation facilitating domain comprising amino acids 874-1110 of SEQ ID NO: 1 of the BoNT/A-XP6A-Galanin and will be subcloned into a pCR2.1 vector using the TOPO® TA cloning method (Invitrogen, Inc, Carlsbad, CA). The forward and reverse oligonucleotide primers used for these reactions are designed to include unique restriction enzyme sites useful for subsequent subcloning steps. The resulting construct will be digested with restriction enzymes that 1) will excise the polynucleotide molecule containing the entire open reading frame encoding the modified BoNT/A-XP6A-Galanin; and 2) will enable this polynucleotide molecule to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). The resulting restriction fragment will be purified by the QIAquick Gel Extraction Kit (QIAGEN, Inc., Valencia, CA), and will be subcloned using a T4 DNA ligase procedure into a pET29 vector. This cloning strategy yielded a pET29 expression construct encoding a BoNT/A-XP6A-Galanin comprising a BoNT/B translocation facilitating domain.

[0437] A similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a BoNT/A-XP6A-Galanin that replaces the region corresponding to the BoNT/A translocation facilitating domain comprising amino acids 874-1110 of SEQ ID NO: 1 with, *e.g.*, a translocation facilitating domain comprising amino acids 869-1111 of BoNT/C1 of SEQ ID NO: 3; a translocation facilitating domain comprising amino acids 865-1098 of BoNT/D of SEQ ID NO: 4; a translocation facilitating domain comprising amino acids 846-1058 of BoNT/E of SEQ ID NO: 5; a translocation facilitating domain comprising amino acids 867-1105 of BoNT/F of SEQ ID NO: 6; a translocation facilitating domain comprising amino acids 866-1105 of BoNT/G of SEQ ID NO: 7; or a translocation facilitating domain comprising amino acids 882-1127 of TeNT of SEQ ID NO: 8. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a BoNT/A-XP6A-Galanin that replaces the region corresponding to the BoNT/A translocation facilitating domain comprising amino acids 874-1110 of SEQ ID NO: 1 with a translocation facilitating domain comprising an enveloped virus fusogenic peptide domain, such as, *e,g,* SEQ ID NO: 194 to SEQ ID NO: 269.

[0438] Likewise, a polynucleotide molecule encoding a Clostridial translocation facilitating domain as described above can be introduced into a polynucleotide molecule encoding BoNT/B-XP6A-Galanin, BoNT/C1-XP6A-Galanin, BoNT/D-XP6A-Galanin, BoNT/E-XP6A-Galanin, BoNT/F-XP6A-Galanin, BoNT/G-XP6A-Galanin, TeNT-XP6A-Galanin, as well as the modified Clostridial toxin-XP6A indicated above comprising SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 88 to SEQ ID NO: 115, SEQ ID NO: 178 to SEQ ID NO: 181, SEQ ID NO: 183 or SEQ ID NO: 184, as well as, amino acids 26-58 of SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115; amino acids 47-58 of SEQ ID NO: 100, SEQ ID NO: 110 or SEQ ID NO: 114; or amino acids 51-58 of SEQ ID NO: 100.

3b. A translocation-translocation facilitating-enzymatic-targeting domain organization.

[0439] A polynucleotide molecule based on BoNT/A-XP6B-Galanin (SEQ ID NO: 193) will be synthesized and cloned into a pUCBHB1 vector as described in Example 1a. This polynucleotide molecule encodes a BoNT/A modified to replace amino acids 1111-1296 of SEQ ID NO: 1, a BoNT/A H_{CC} targeting domain, with SEQ ID NO: 72, a Galanin targeting domain, and has the general domain arrangement of FIG. 6B. If so desired, expression optimization to a different organism, such as, *e.g.*, a bacteria, a yeast strain, an insect cell-line or a mammalian cell line, can be done as described above, see, *e.g.*, Steward, *supra*, (Feb. 2, 2006); and Steward, *supra*, (Feb. 16, 2006).

[0440] Likewise, a similar cloning strategy will be used to make pUCBHB1 cloning constructs comprising a polynucleotide molecule encoding a modified BoNT/A-XP6B with an altered targeting domain such as, *e.g*, altered targeting domains SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184; and altered targeting domains requiring a free amino-terminal amino acid like SEQ ID NO: 88 to SEQ ID NO: 115 and SEQ ID NO: 178 to SEQ ID NO: 181, as well as, amino acids 26-58 of SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115; amino acids 47-58 of SEQ ID NO: 100, SEQ ID NO: 110 or SEQ ID NO: 114; or amino acids 51-58 of SEQ ID NO: 100. In addition, similar cloning strategy will be used to produce a modified Clostridial toxin-XP6B, such as, *e.g.*, BoNT/B-XP6B, BoNT/C1-XP6B, BoNT/D-XP6B, BoNT/E-XP6B, BoNT/F-XP6B, BoNT/G-XP6B or TeNT-XP6B, to comprise an altered targeting domain comprising any one of SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 88 to SEQ ID NO: 115, SEQ ID NO: 183 or SEQ ID NO: 184, or amino acids 26-58 of SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115; amino acids 47-58 of SEQ ID NO: 100, SEQ ID NO: 110 or SEQ ID NO: 114; or amino acids 51-58 of SEQ ID NO: 100.

[0441] To construct pET29/BoNT/A-XP6B-Galanin, a similar cloning strategy will be used as described in Example 1a. This cloning strategy will yield a pET29 expression construct comprising the polynucleotide molecule encoding the BoNT/A-XP6B-Galanin operably-linked to a carboxyl terminal polyhistidine affinity binding peptide. A similar cloning strategy will be used to make pET29 expression constructs for other modified Clostridial toxin-XP6B-Galanin toxins, such as, *e.g.*, BoNT/B-XP6B-Galanin, BoNT/C1-XP6B-Galanin, BoNT/D-XP6B-Galanin, BoNT/E-XP6B-Galanin, BoNT/F-XP6B-Galanin, BoNT/G-XP6B-Galanin or TeNT-XP6B-Galanin. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-XP6B with an altered targeting domain such as, *e.g*, altered targeting domains SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 183 or SEQ ID NO: 184; and altered targeting domains requiring a free amino-terminal amino acid like SEQ ID NO: 88 to SEQ ID NO: 115 and SEQ ID NO: 178 to SEQ ID NO: 181, as well as, amino acids 26-58 of SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115; amino acids 47-58 of SEQ ID NO: 100, SEQ ID NO: 110 or SEQ ID NO: 114; or amino acids 51-58 of SEQ ID NO: 100.

[0442] To construct a BoNT/A-XP6B-Galanin that will replace the BoNT/A translocation facilitating domain with another Clostridial toxin translocation facilitating domain, a similar cloning strategy using SOE-PCR will be used as described in Example 1a. This cloning strategy yielded a pET29 expression construct encoding a BoNT/A-XP6B-Galanin comprising a BoNT/B translocation facilitating domain, a BoNT/C1 translocation facilitating domain, a BoNT/D translocation facilitating domain, a BoNT/E translocation facilitating domain, a BoNT/F translocation facilitating domain, a BoNT/G translocation facilitating domain and a TeNT translocation facilitating domain, as well as, a translocation facilitating domain comprising an enveloped virus fusogenic peptide domain, such as, *e,g,* SEQ ID NO: 194 to SEQ ID NO: 269.

[0443] Likewise, a polynucleotide molecule encoding a Clostridial translocation facilitating domain as described above can be introduced into a polynucleotide molecule encoding BoNT/B-XP6B-Galanin, BoNT/C1-XP6B-Galanin, BoNT/D-XP6B-Galanin, BoNT/E-XP6B-Galanin, BoNT/F-XP6B-Galanin, BoNT/G-XP6B-Galanin, TeNT-XP6B-Galanin, as well as the modified Clostridial toxin-XP6B indicated above comprising SEQ ID NO: 62 to SEQ ID NO: 71, SEQ ID NO: 88 to SEQ ID NO: 115, SEQ ID NO: 178 to SEQ ID NO: 181, SEQ ID NO: 183 or SEQ ID NO: 184, as well as, amino acids 26-58 of SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114 or SEQ ID NO: 115; amino acids 47-58 of SEQ ID NO: 100, SEQ ID NO: 110 or SEQ ID NO: 114; or amino acids 51-58 of SEQ ID NO: 100.

Example 4

Expression of Modified Clostridial Toxins in a Bacterial Cell

[0444] The following example illustrates a procedure useful for expressing any of the modified Clostridial toxins disclosed in the present specification in a bacterial cell.

[0445] An expression construct, such as, *e.g.*, any of the expression constructs in Examples 1-3, will be introduced into chemically competent *E. coli* BL21 (DE3) cells (Invitrogen, Inc, Carlsbad, CA) using a heat-shock transformation protocol. The heat-shock reaction will be plated onto 1.5% Luria-Bertani agar plates (pH 7.0) containing 50 µg/mL of Kanamycin and will be placed in a 37 °C incubator for overnight growth. Kanamycin-resistant colonies of transformed *E. coli* containing the expression construct will be used to inoculate a baffled flask containing 3.0 mL of PA-0.5G media containing 50 µg/mL of Kanamycin which will then placed in a 37 °C incubator, shaking at 250 rpm, for overnight growth. The resulting overnight starter culture will be used to inoculate a 3 L baffled flask containing ZYP-5052 autoinducing media containing 50 µg/mL of Kanamycin at a dilution of 1:1000. Culture volumes will ranged from about 600 mL (20% flask volume) to about 750 mL (25% flask volume). These cultures will be grown in a 37 °C incubator shaking at 250 rpm for approximately 5.5 hours and will be then transferred to a 16 °C incubator shaking at 250 rpm for overnight expression. Cells will be harvested by centrifugation (4,000 rpm at 4 °C for 20-30 minutes) and will be used immediately, or will be stored dry at –80 °C until needed.

Example 5

Purification and Quantification of Modified Clostridial Toxins

[0446] The following example illustrates methods useful for purification and quantification of any modified Clostridial toxins disclosed in the present specification.

[0447] For immobilized metal affinity chromatography (IMAC) protein purification, *E. coli* BL21 (DE3) cell pellets used to express a modified Clostridial toxin, as described in Example 4, will be resuspended in Column Binding Buffer (25 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 7.8; 500 mM sodium chloride; 10 mM imidazole; 2x Protease Inhibitor Cocktail Set III (EMD Biosciences-Calbiochem, San Diego CA); 5 units/mL of Benzonase (EMD Biosciences-Novagen, Madison, WI); 0.1% (v/v) Triton-X® 100, 4-octylphenol polyethoxylate; 10% (v/v) glycerol), and will then be transferred to a cold Oakridge centrifuge tube. The cell suspension will be sonicated on ice (10-12 pulses of 10 seconds at 40% amplitude with 60 seconds cooling intervals on a Branson Digital Sonifier) in order to lyse the cells and then is centrifuged (16,000 rpm at 4 °C for 20 minutes) to clarify the lysate. An immobilized metal affinity chromatography column will be prepared using a 20 mL Econo-Pac column support (Bio-Rad Laboratories, Hercules, CA) packed with 2.5-5.0 mL of TALON™ SuperFlow Co^{2+} affinity resin (BD Biosciences-Clontech, Palo Alto, CA), which will then be equilibrated by rinsing with 5 column volumes of deionized, distilled water, followed by 5 column volumes of Column Binding Buffer. The clarified lysate

will be applied slowly to the equilibrated column by gravity flow (approximately 0.25-0.3 mL/minute). The column will then be washed with 5 column volumes of Column Wash Buffer (*N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 7.8; 500 mM sodium chloride; 10 mM imidazole; 0.1% (v/v) Triton-X® 100, 4-octylphenol polyethoxylate; 10% (v/v) glycerol). The modified Clostridial toxin will be eluted with 20-30 mL of Column Elution Buffer (25 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 7.8; 500 mM sodium chloride; 500 mM imidazole; 0.1% (v/v) Triton-X® 100, 4-octylphenol polyethoxylate; 10% (v/v) glycerol) and will be collected in approximately twelve 1 mL fractions. The amount of modified Clostridial toxin contained in each elution fraction will be determined by a Bradford dye assay. In this procedure, 20 µL aliquots of each 1.0 mL fraction will be combined with 200 µL of Bio-Rad Protein Reagent (Bio-Rad Laboratories, Hercules, CA), diluted 1 to 4 with deionized, distilled water, and then the intensity of the colorimetric signal will be measured using a spectrophotometer. The five fractions with the strongest signal will be considered the elution peak and will be combined together. Total protein yield will be determined by estimating the total protein concentration of the pooled peak elution fractions using bovine gamma globulin as a standard (Bio-Rad Laboratories, Hercules, CA).

[0448] For purification of a modified Clostridial toxin using a FPLC desalting column, a HiPrep™ 26/10 size exclusion column (Amersham Biosciences, Piscataway, NJ) will be pre-equilibrated with 80 mL of 4 °C Column Buffer (50 mM sodium phosphate, pH 6.5). After the column is equilibrated, a modified Clostridial toxin sample will be applied to the size exclusion column with an isocratic mobile phase of 4 °C Column Buffer and at a flow rate of 10 mL/minute using a BioLogic DuoFlow chromatography system (Bio-Rad Laboratories, Hercules, CA). The desalted modified Clostridial toxin sample will be collected as a single fraction of approximately 7-12 mL.

[0449] For purification of a modified Clostridial toxin using a FPLC ion exchange column, a modified Clostridial toxin sample that has been desalted following elution from an IMAC column will be applied to a 1 mL Q1™ anion exchange column (Bio-Rad Laboratories, Hercules, CA) using a BioLogic DuoFlow chromatography system (Bio-Rad Laboratories, Hercules, CA). The sample will be applied to the column in 4 °C Column Buffer (50 mM sodium phosphate, pH 6.5) and will be eluted by linear gradient with 4 °C Elution Buffer (50 mM sodium phosphate, 1 M sodium chloride, pH 6.5) as follows: step 1, 5.0 mL of 5% Elution Buffer at a flow rate of 1 mL/minute; step 2, 20.0 mL of 5-30% Elution Buffer at a flow rate of 1 mL/minute; step 3, 2.0 mL of 50% Elution Buffer at a flow rate of 1.0 mL/minute; step 4, 4.0 mL of 100% Elution Buffer at a flow rate of 1.0 mL/minute; and step 5, 5.0 mL of 0% Elution Buffer at a flow rate of 1.0 mL/minute. Elution of modified Clostridial toxin from the column will be monitored at 280, 260, and 214 nm, and peaks absorbing above a minimum threshold (0.01 au) at 280 nm will be collected. Most of the modified Clostridial toxin will be eluted at a sodium chloride concentration of approximately 100 to 200 mM. Average total yields of modified Clostridial toxin will be determined by a Bradford assay.

[0450] Expression of a modified Clostridial toxin will be analyzed by polyacrylamide gel electrophoresis. Samples purified using the procedure described above are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and will be separated by MOPS polyacrylamide gel electrophoresis using NuPAGE® Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen, Inc, Carlsbad, CA) under denaturing, reducing conditions. Gels will be stained with SYPRO® Ruby (Bio-Rad Laboratories, Hercules, CA) and the separated polypeptides will be imaged using a Fluor-S MAX MultiImager (Bio-Rad Laboratories, Hercules, CA) for quantification of modified Clostridial toxin expression levels. The size and amount of modified Clostridial toxin will be determined by comparison to MagicMark™ protein molecular weight standards (Invitrogen, Inc, Carlsbad, CA).

[0451] Expression of modified Clostridial toxin will also be analyzed by Western blot analysis. Protein samples purified using the procedure described above will be added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and will be separated by MOPS polyacrylamide gel electrophoresis using NuPAGE® Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen, Inc, Carlsbad, CA) under denaturing, reducing conditions. Separated polypeptides will be transferred from the gel onto polyvinylidene fluoride (PVDF) membranes (Invitrogen, Inc, Carlsbad, CA) by Western blotting using a Trans-Blot® SD semi-dry electrophoretic transfer cell apparatus (Bio-Rad Laboratories, Hercules, CA). PVDF membranes will be blocked by incubating at room temperature for 2 hours in a solution containing 25 mM Tris-Buffered Saline (25 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloric acid (Tris-HCl)(pH 7.4), 137 mM sodium chloride, 2.7 mM potassium chloride), 0.1% TWEEN-20®, polyoxyethylene (20) sorbitan monolaureate, 2% bovine serum albumin, 5% nonfat dry milk. Blocked membranes will be incubated at 4 °C for overnight in Tris-Buffered Saline TWEEN-20® (25 mM Tris-Buffered Saline, 0.1% TWEEN-20®, polyoxyethylene (20) sorbitan monolaureate) containing appropriate primary antibodies as a probe. Primary antibody probed blots will be washed three times for 15 minutes each time in Tris-Buffered Saline TWEEN-20®. Washed membranes will be incubated at room temperature for 2 hours in Tris-Buffered Saline TWEEN-20® containing an appropriate immunoglobulin G antibody conjugated to horseradish peroxidase as a secondary antibody. Secondary antibody-probed blots will be washed three times for 15 minutes each time in Tris-Buffered Saline TWEEN-20®. Signal detection of the labeled modified Clostridial toxin will be visualized using the ECL Plus™ Western Blot Detection System (Amersham Biosciences, Piscataway, NJ) and will be imaged with a Typhoon 9410 Variable Mode Imager (Amersham Biosciences, Piscataway, NJ) for quantification of modified Clostridial toxin expression levels.

[0452] Although aspects of the present invention have been described with reference to the disclosed embodiments, one skilled in the art will readily appreciate that the specific examples disclosed are only illustrative of these aspects and in no way limit the present invention. Various modifications can be made without departing from the spirit of the present invention.

What is claimed is

1. A modified Clostridial toxin comprising:

    a) a Clostridial toxin enzymatic domain capable of executing an enzymatic target modification step of a Clostridial toxin intoxication process;
    b) a Clostridial toxin translocation domain capable of executing a translocation step of a Clostridial toxin intoxication process;
    c) a translocation facilitating domain capable of facilitating a translocation step of a Clostridial toxin intoxication process;
    d) an altered targeting domain capable of selectively binding a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell and executing a cell binding step of a Clostridial toxin intoxication process; and
    e) a protease cleavage site

    wherein cleavage of the protease cleavage site converts the single-chain form of the modified Clostridial toxin into the di-chain form.

2. The modified Clostridial toxin according to Claim 1, wherein the modified Clostridial toxin comprises a linear amino-to-carboxyl single polypeptide order of 1) the Clostridial toxin enzymatic domain, the protease cleavage site, the Clostridial toxin translocation domain, the translocation facilitating domain and the altered targeting domain, 2) the Clostridial toxin enzymatic domain, the protease cleavage site, the altered targeting domain, the Clostridial toxin translocation domain and the translocation facilitating domain, 3) the altered targeting domain, the Clostridial toxin translocation domain, the translocation facilitating domain, the protease cleavage site and the Clostridial toxin enzymatic domain, 4) the altered targeting domain, the Clostridial toxin enzymatic domain, the protease cleavage site, the Clostridial toxin translocation domain and the translocation facilitating domain, 5) the Clostridial toxin translocation domain, the translocation facilitating domain, the protease cleavage site, the Clostridial toxin enzymatic domain and the altered targeting domain, or 6) the Clostridial toxin translocation domain, the translocation facilitating domain, the protease cleavage site, the altered targeting domain and the Clostridial toxin enzymatic domain.

3. The modified Clostridial toxin according to Claim 1, wherein the translocation facilitating domain is a Clostridial toxin translocation facilitating domain.

4. The modified Clostridial toxin according to Claim 1, wherein the translocation facilitating domain is an enveloped virus fusogenic peptide domain.

5. The modified Clostridial toxin according to Claim 1, wherein the altered targeting domain is selected from the group consisting of an opioid, a melanocortin, a galanin, a granin, a tachykinin, a cholecystokinin, a Neuropeptide Y related, a kinin peptide, a PAR peptide, a corticotropin-releasing hormone, a thyrotropin-releasing hormone and a somatostatin.

6. The modified Clostridial toxin according to Claim 5, wherein the opioid is an enkephalin, a BAM22 peptide, an endomorphin, an endorphin, a dynorphin, a nociceptin or a hemorphin.

7. The modified Clostridial toxin according to Claim 5, wherein the melanocortin is an α-melanocyte stimulating hormones (α-MSH), a β-melanocyte stimulating hormones (β-MSH), a γ-melanocyte stimulating hormones (γ-MSH), an adrenocorticotropin (ACTH), a Corticotropin-like intermediary peptide (CLIP), a β-lipotropin (β-LPH) or a γ-lipotropin (γ-LPH).

8. The modified Clostridial toxin according to Claim 5, wherein the galanin selected from the group consisting of a galanin or a galanin message-associated peptide (GMAP).

9. The modified Clostridial toxin according to Claim 5, wherein the grainin is a chromogranin A peptide, a chromogranin B peptide or a chromogranin C peptide.

10. The modified Clostridial toxin according to Claim 5, wherein the tachykinin peptide is a Substance P, a neuropeptide K (NPK), a neuropeptide gamma, a neurokinin A, a neurokinin B, a hemokinin and a endokinin.

11. The modified Clostridial toxin according to Claim 5, wherein the cholecystokinin peptide is a cholecystokinin 58, a cholecystokinin 39, a cholecystokinin 33, a cholecystokinin 12 or a cholecystokinin 8.

12. The modified Clostridial toxin according to Claim 5, wherein the Neuropeptide Y related peptide is a Neuropeptide Y (NPY), a Peptide YY (PYY), a Pancreatic peptide (PP) or a Pancreatic icosapeptide (PIP).

13. The modified Clostridial toxin according to Claim 5, wherein the kinin peptide is a bradykinin, a kallidin, a desArg^{9} bradykinin or a desArg^{10} bradykinin.

14. The modified Clostridial toxin according to Claim 5, wherein the PAR peptide is a PAR1 peptide, a PAR2 peptide, a PAR3 peptide or a PAR4 peptide.

15. The modified Clostridial toxin according to Claim 1, wherein the protease cleavage site is an endogenous Clostridial toxin di-chain loop protease cleavage site or an exogenous cleavage site.

16. A polynucleotide molecule encoding a modified Clostridial toxin according to Claim 1.

17. The polynucleotide molecule according to Claim 16, wherein the polynucleotide molecule comprises an expression vector.

18. A method of producing a modified Clostridial toxin comprising the step of expressing a polynucleotide molecule encoding a modified Clostridial toxin in a cell, the polynucleotide molecule according to Claim 1.

19. A method of producing a modified Clostridial toxin comprising the steps of:

a) introducing into a cell a polynucleotide molecule encoding a modified Clostridial toxin, the polynucleotide according to Claim 1; and

b) expressing the modified Clostridial toxin encoded by the polynucleotide molecule.

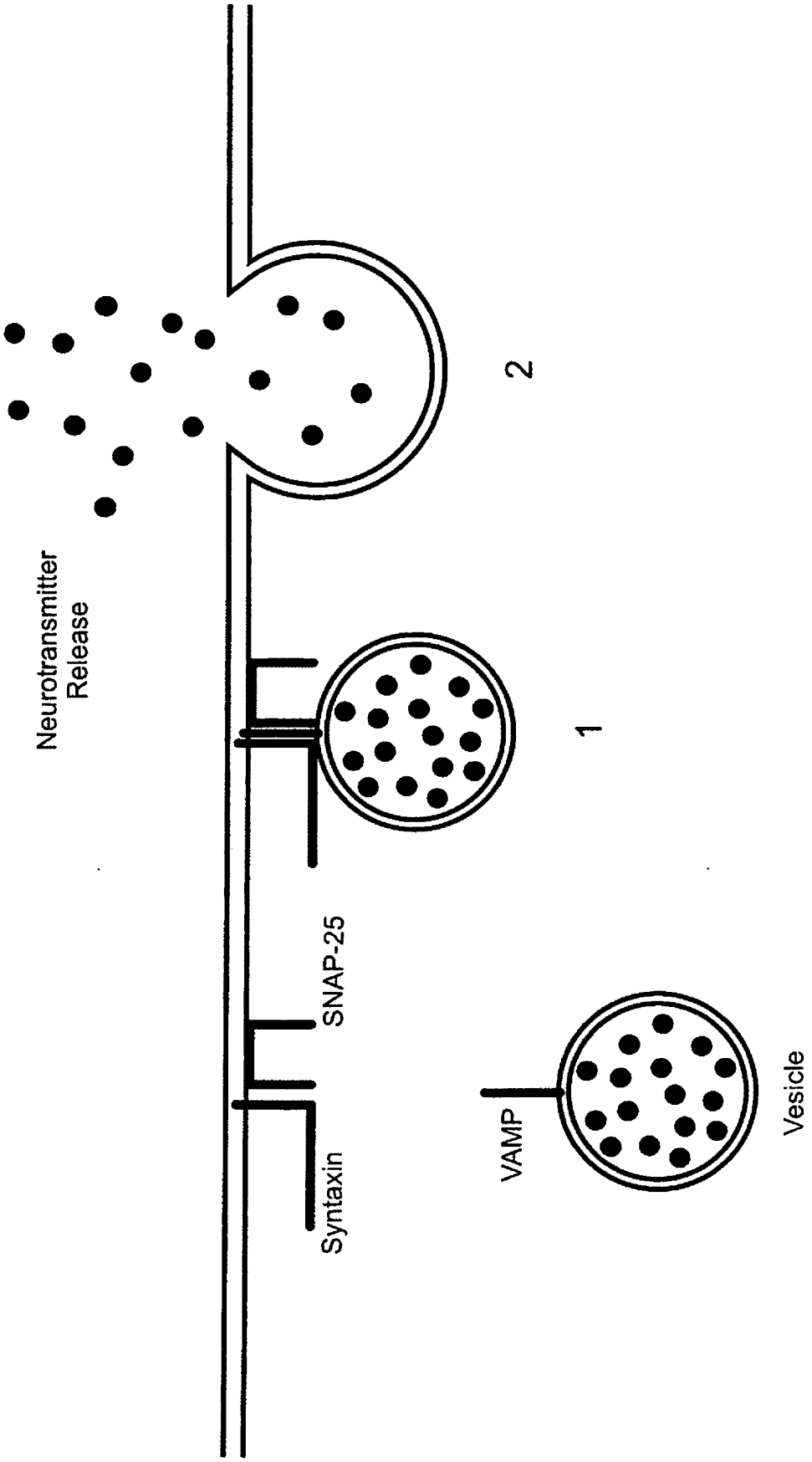
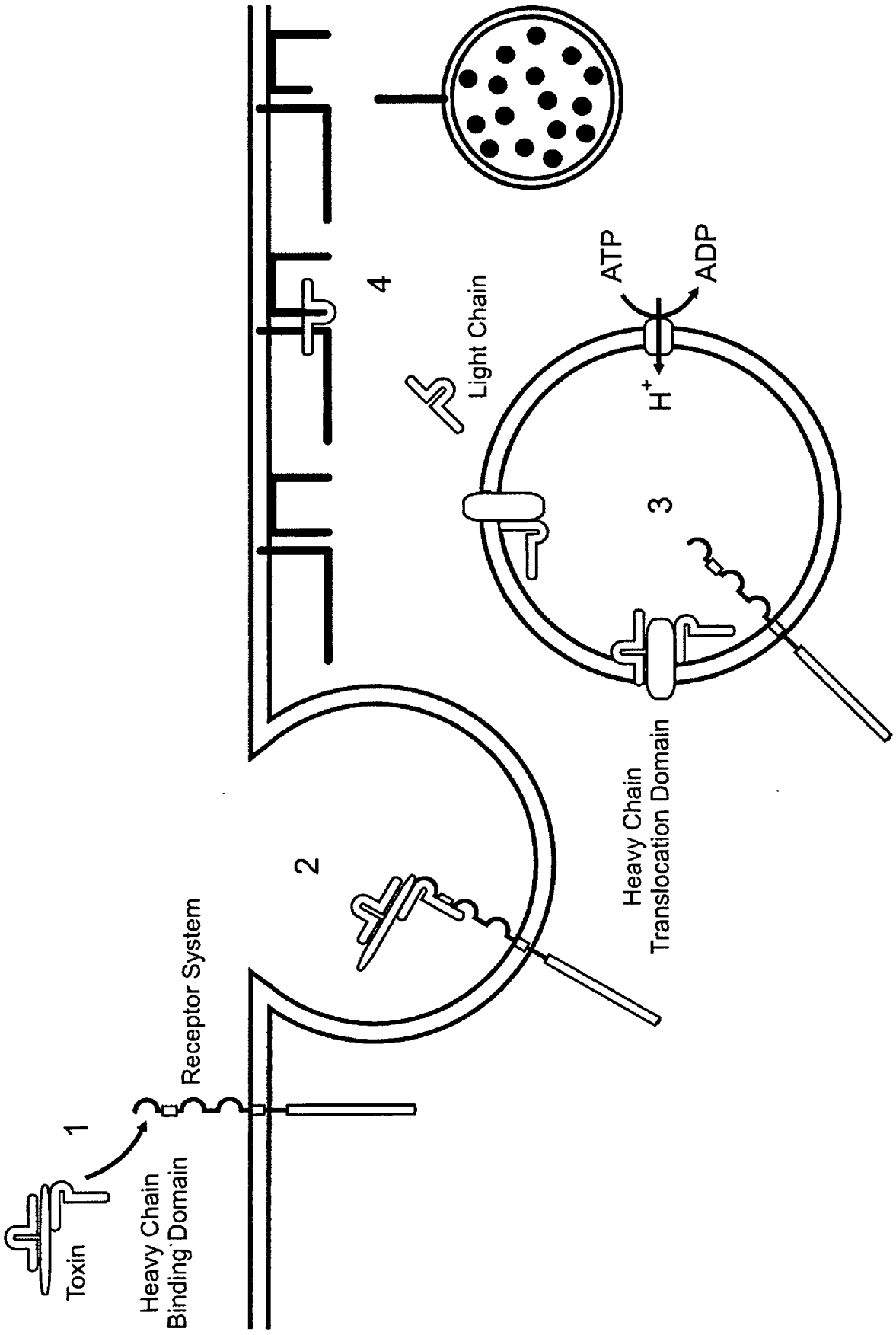

Neurotransmitter Release
2
1
SNAP-25
Syntaxin
VAMP
Vesicle

FIG. 1A.

FIG. 1B.


Toxin
1
Heavy Chain
Binding Domain
Receptor System
2
Heavy Chain
Translocation Domain
3
ATP
ADP
H+
4
Light Chain

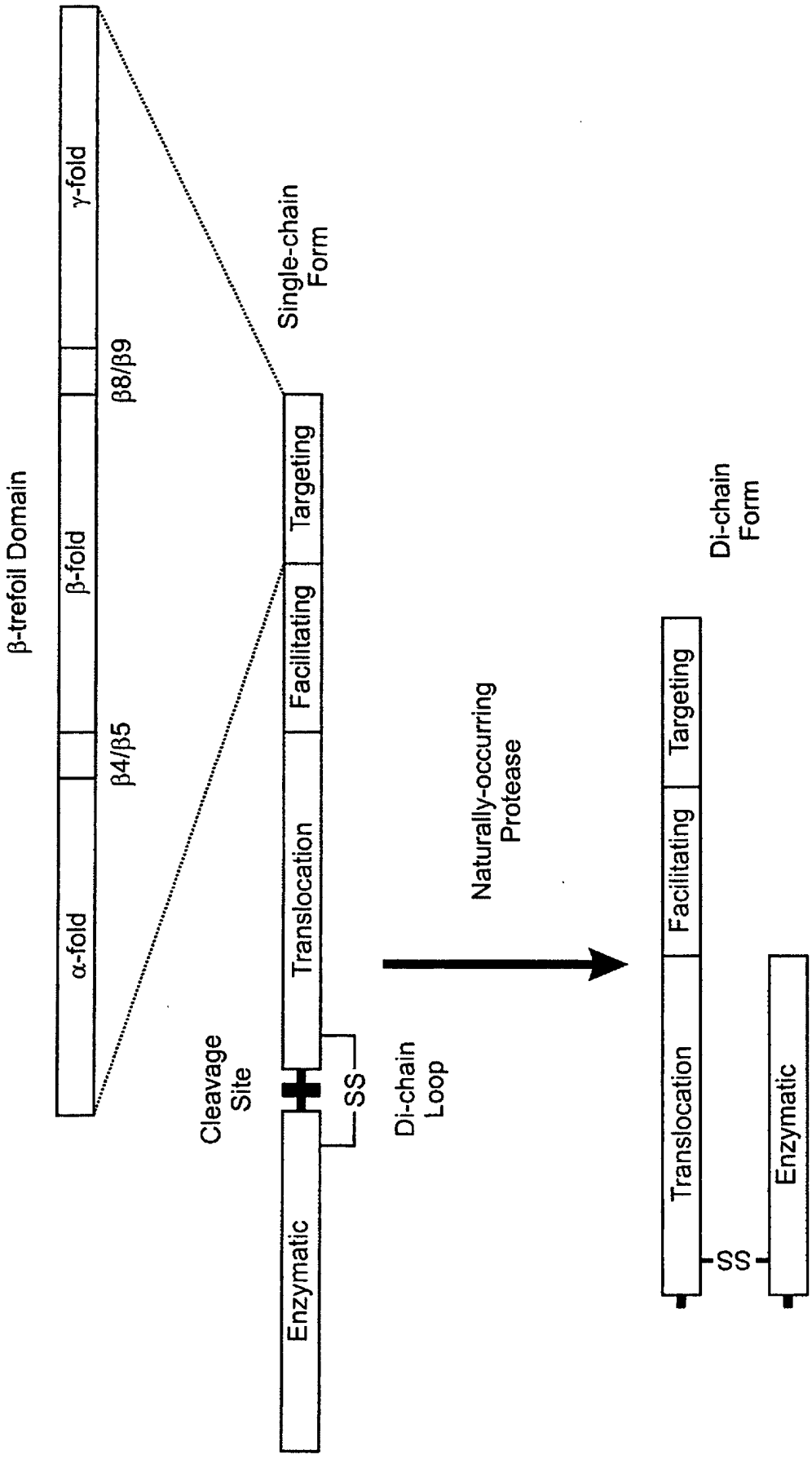

β-trefoil Domain
α-fold
β4/β5
β-fold
β8/β9
γ-fold
Single-chain Form
Translocation
Facilitating
Targeting
Cleavage Site
SS
Enzymatic
Di-chain Loop
Naturally-occurring Protease
Di-chain Form
Translocation
Facilitating
Targeting
SS
Enzymatic

FIG. 2.

FIG. 3.

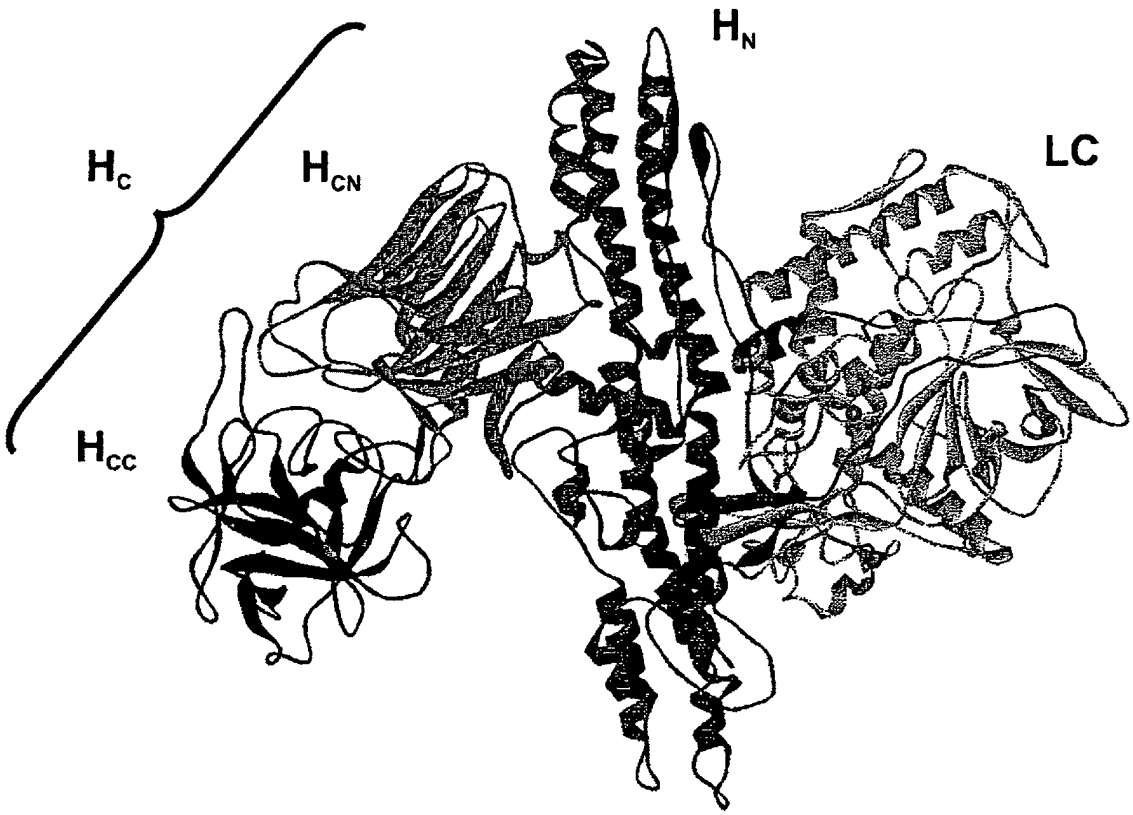


FIG. 4A.

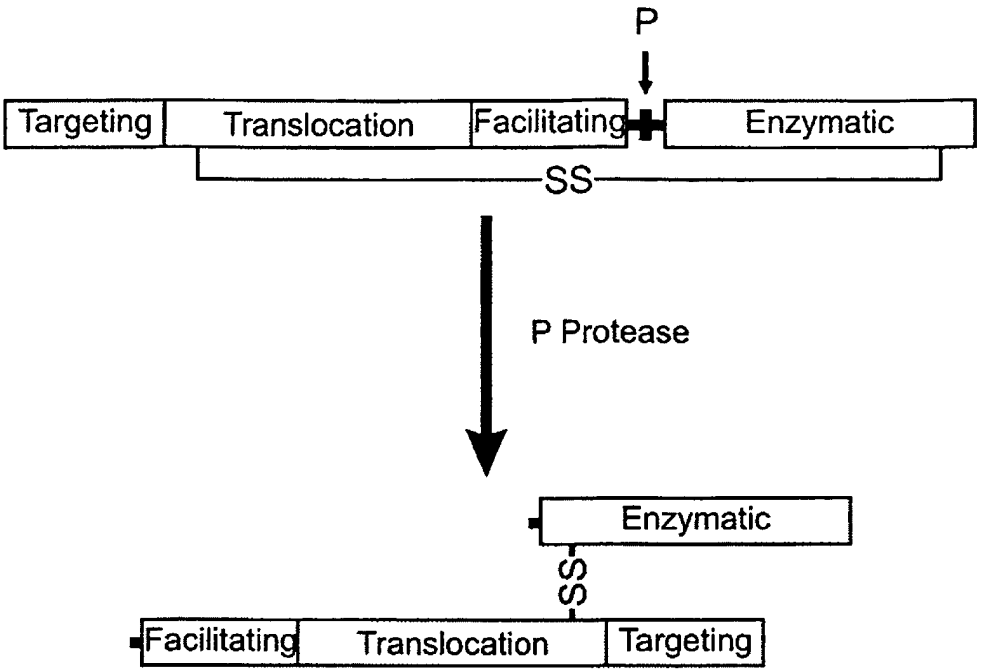

P
Targeting
Translocation
Facilitating
Enzymatic
SS
P Protease
Enzymatic
SS
Facilitating
Translocation
Targeting

FIG. 4B.

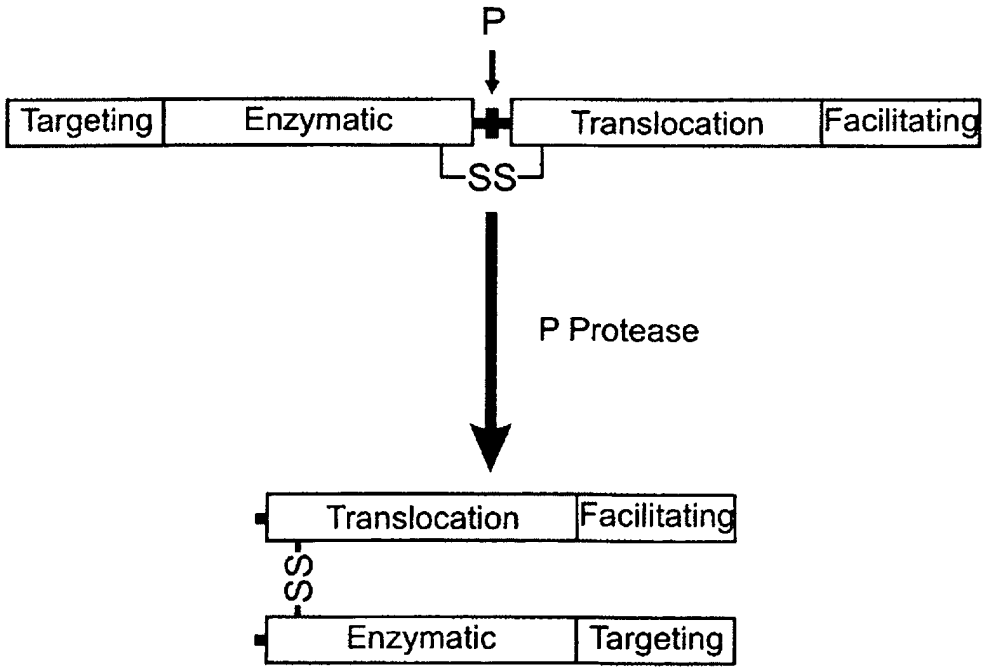
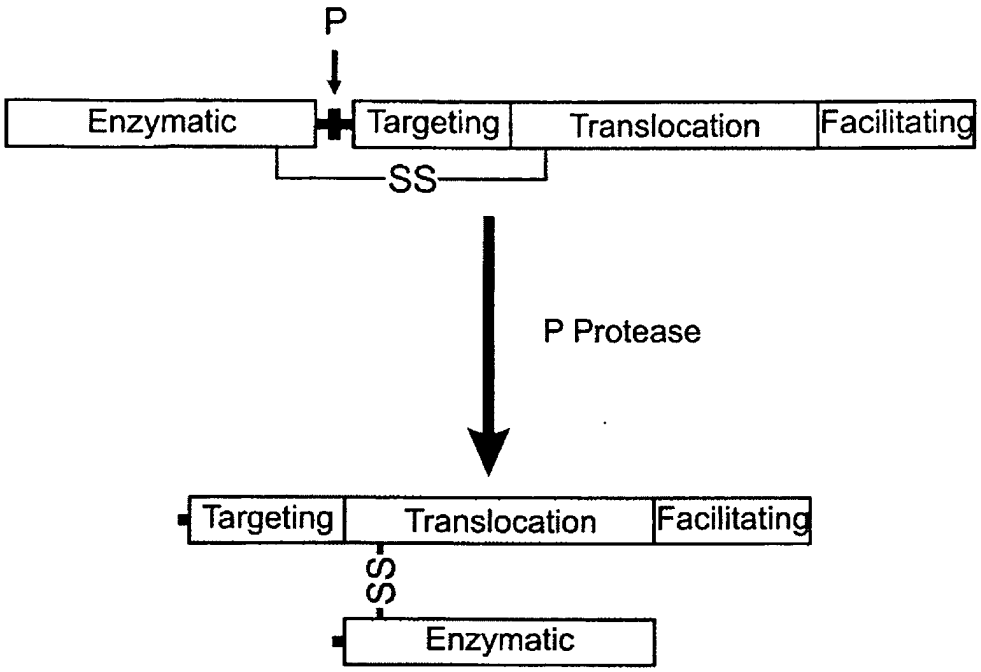

P
Targeting
Enzymatic
Translocation
Facilitating
SS
P Protease
Translocation
Facilitating
SS
Enzymatic
Targeting

FIG. 5A.


P
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Targeting
Translocation
Facilitating
SS
P Protease
Targeting
Translocation
Facilitating
SS
Enzymatic

FIG. 5B.

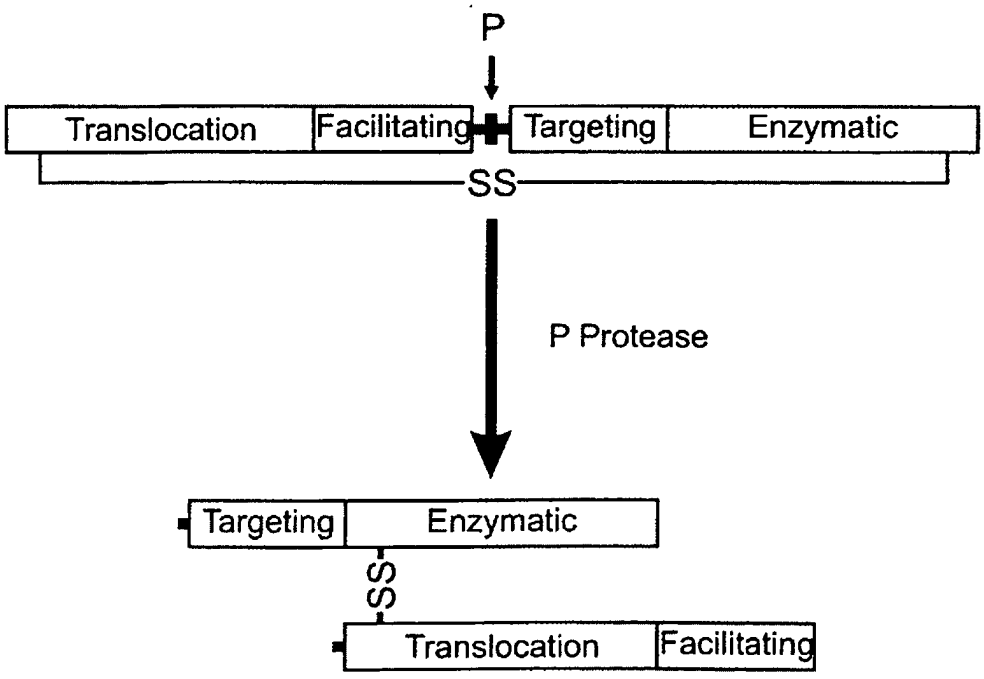
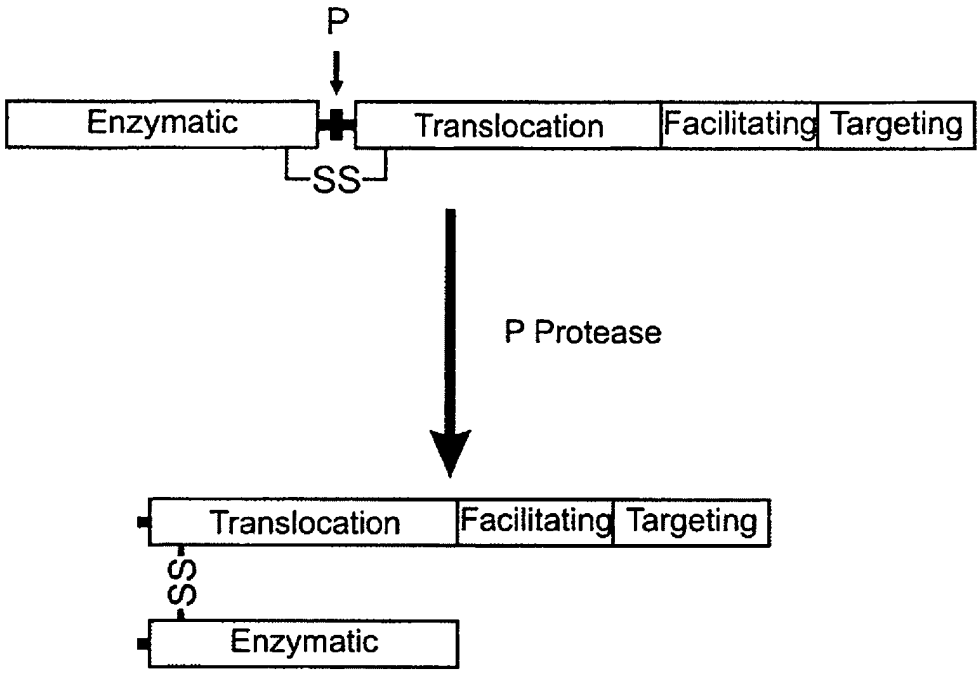

P
Translocation
Facilitating
Targeting
Enzymatic
SS
P Protease
Targeting
Enzymatic
SS
Translocation
Facilitating

FIG. 6A.


P
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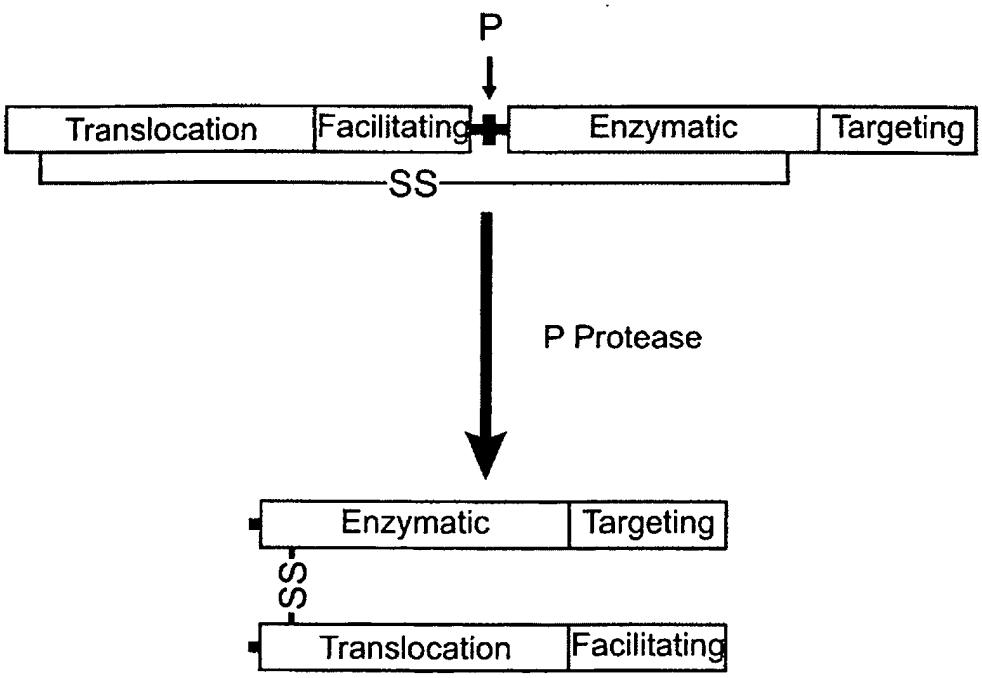
Translocation
Facilitating
Targeting
SS
P Protease
Translocation
Facilitating
Targeting
SS
Enzymatic

FIG. 6B.


P
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Facilitating
Enzymatic
Targeting
SS
P Protease
Enzymatic
Targeting
SS
Translocation
Facilitating

FIG. 7A.

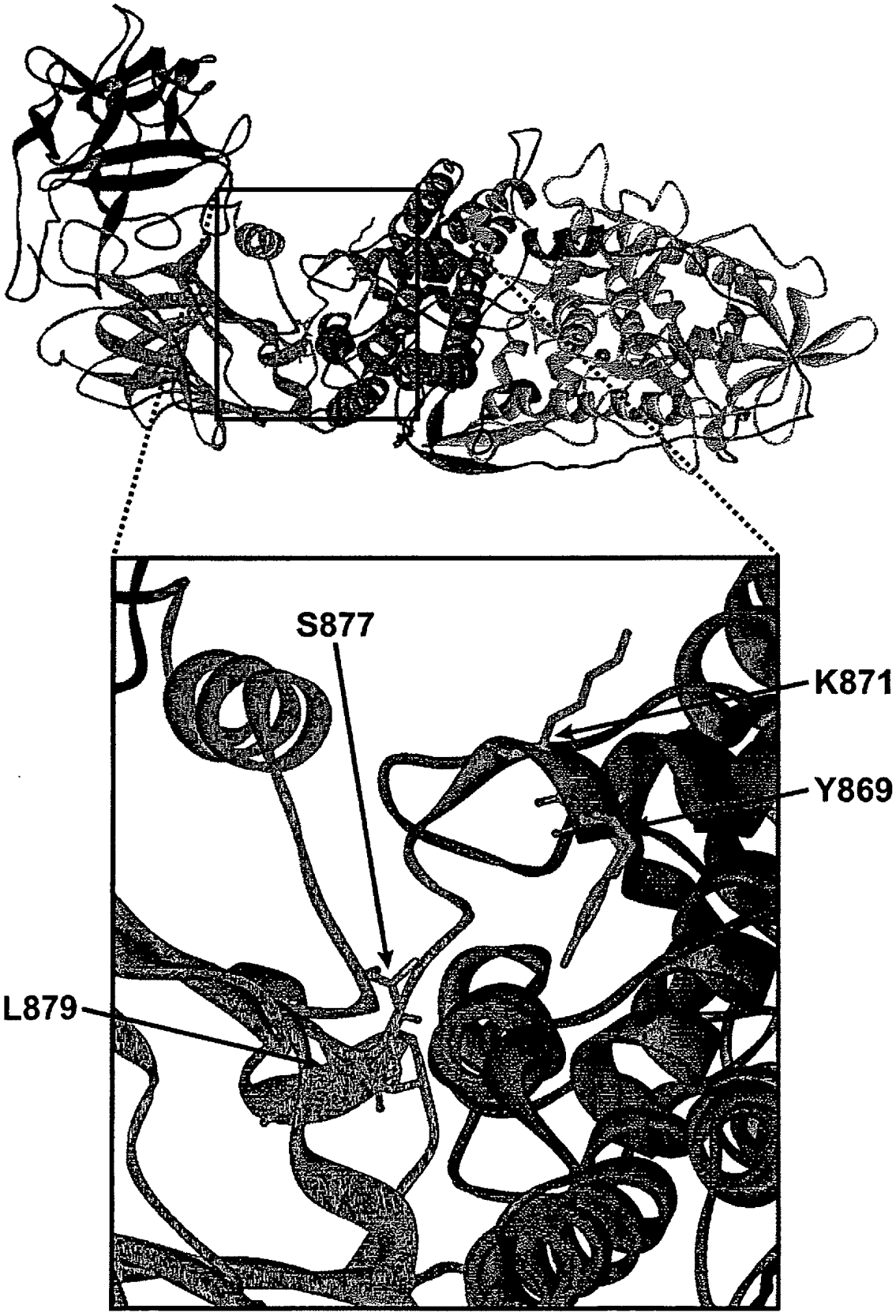
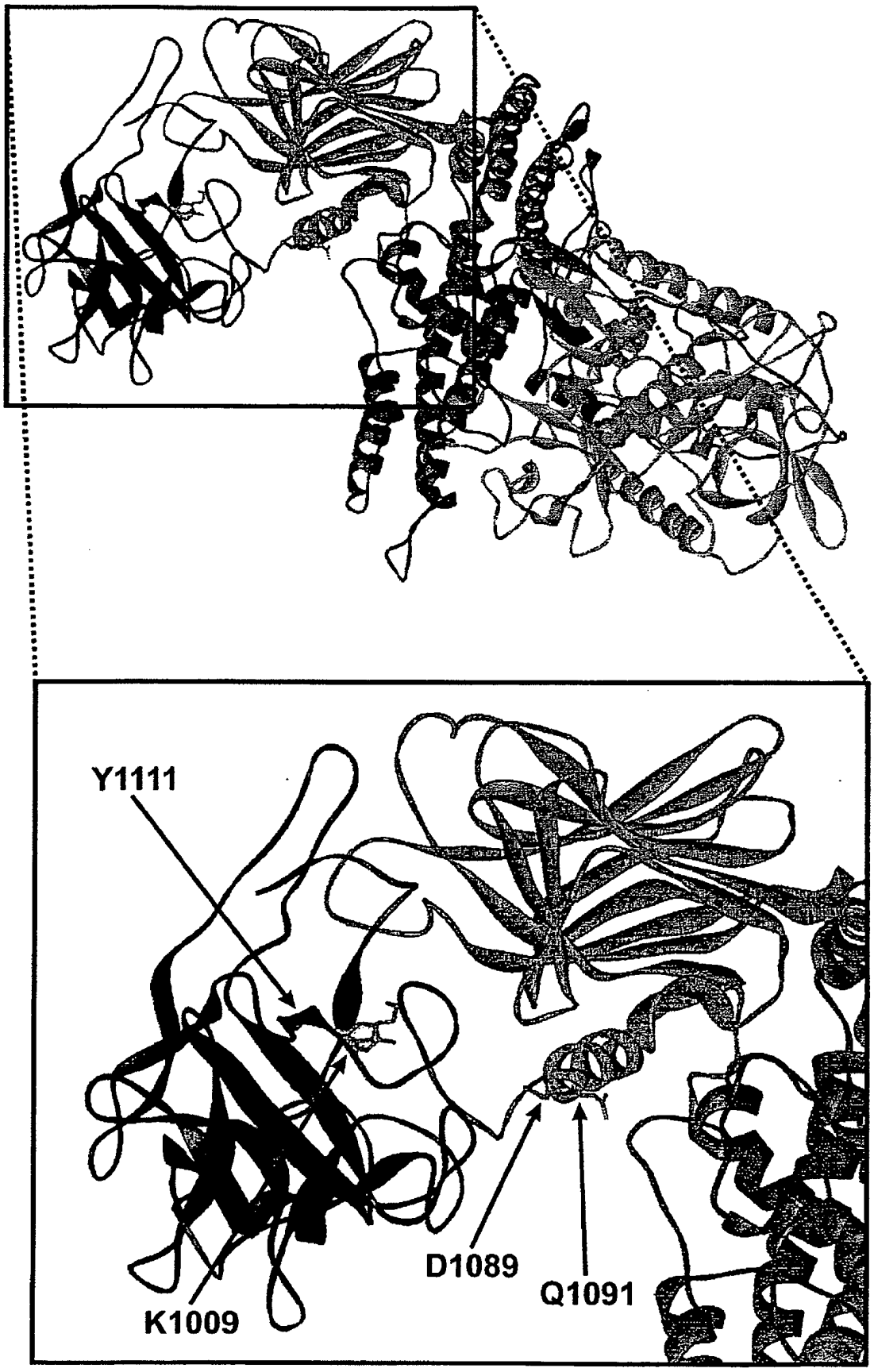
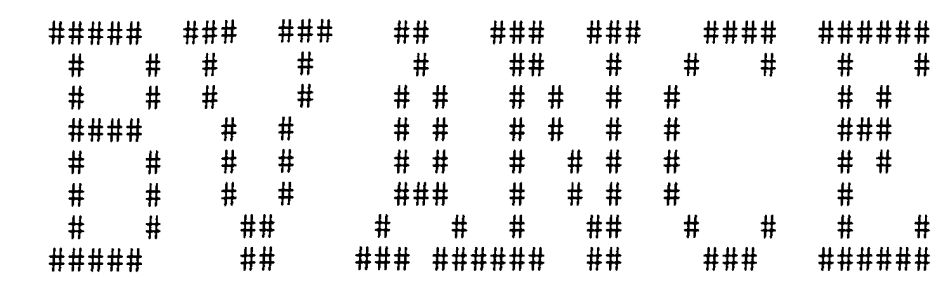


FIG. 7B.





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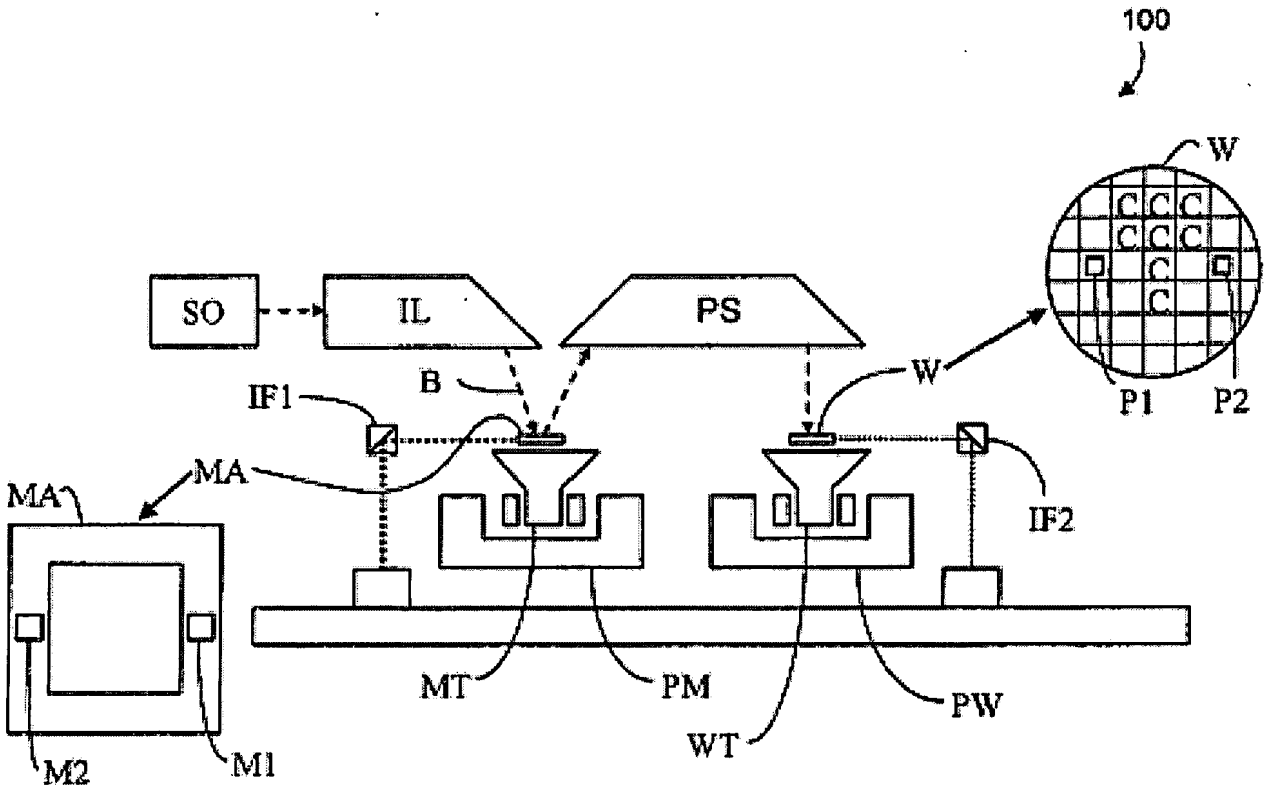
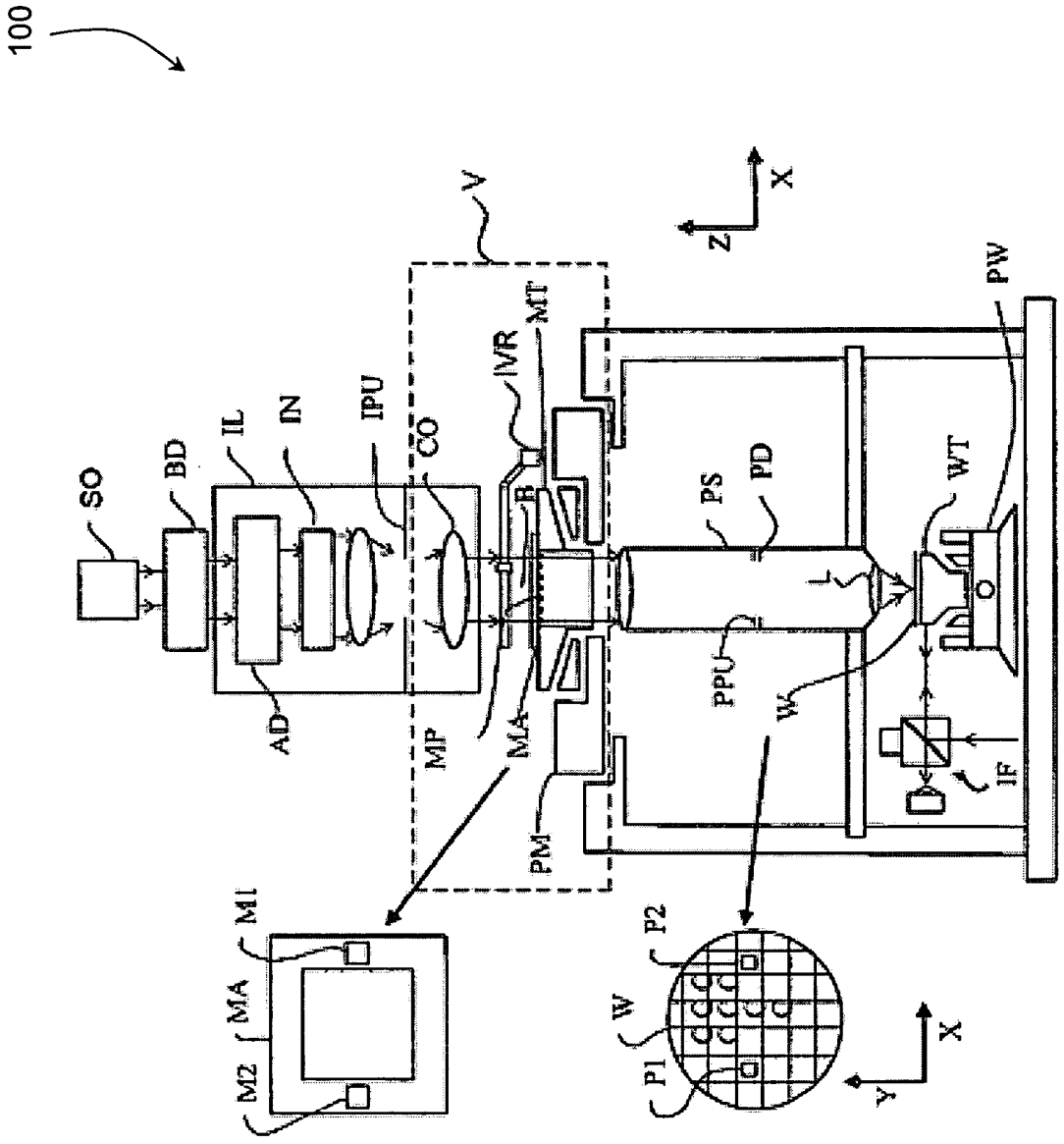


FIG. 1A


100
SO
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IL
IN
IPU
CO
V
IVR
MT
PW
Z
X
PS
PD
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PPU
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IF
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M1
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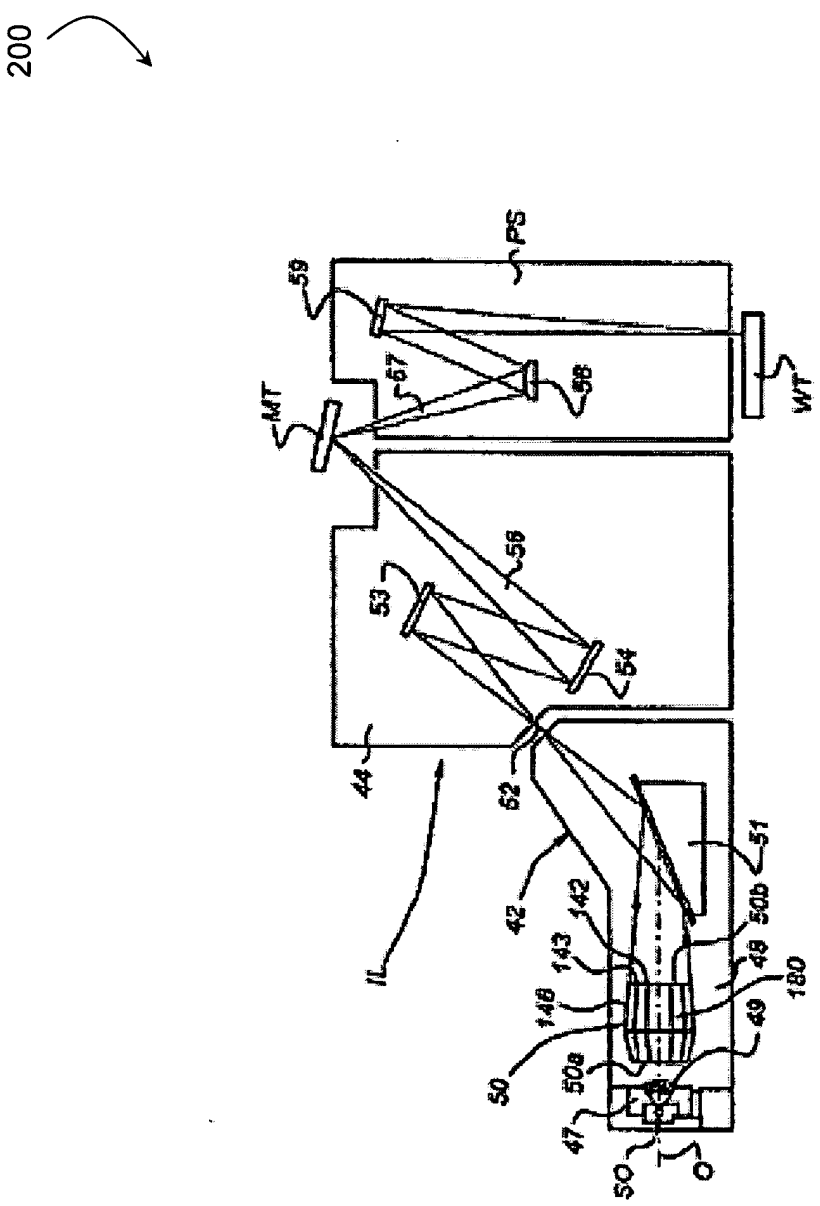
M2
P2
W
P1
X
Y

FIG. 1B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PS
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57
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MT
WT
56
53
54
44
52
42
51
142
143
148
50b
49
180
49
50
50a
47
50
T
O
IL

FIG. 2

FIG. 3

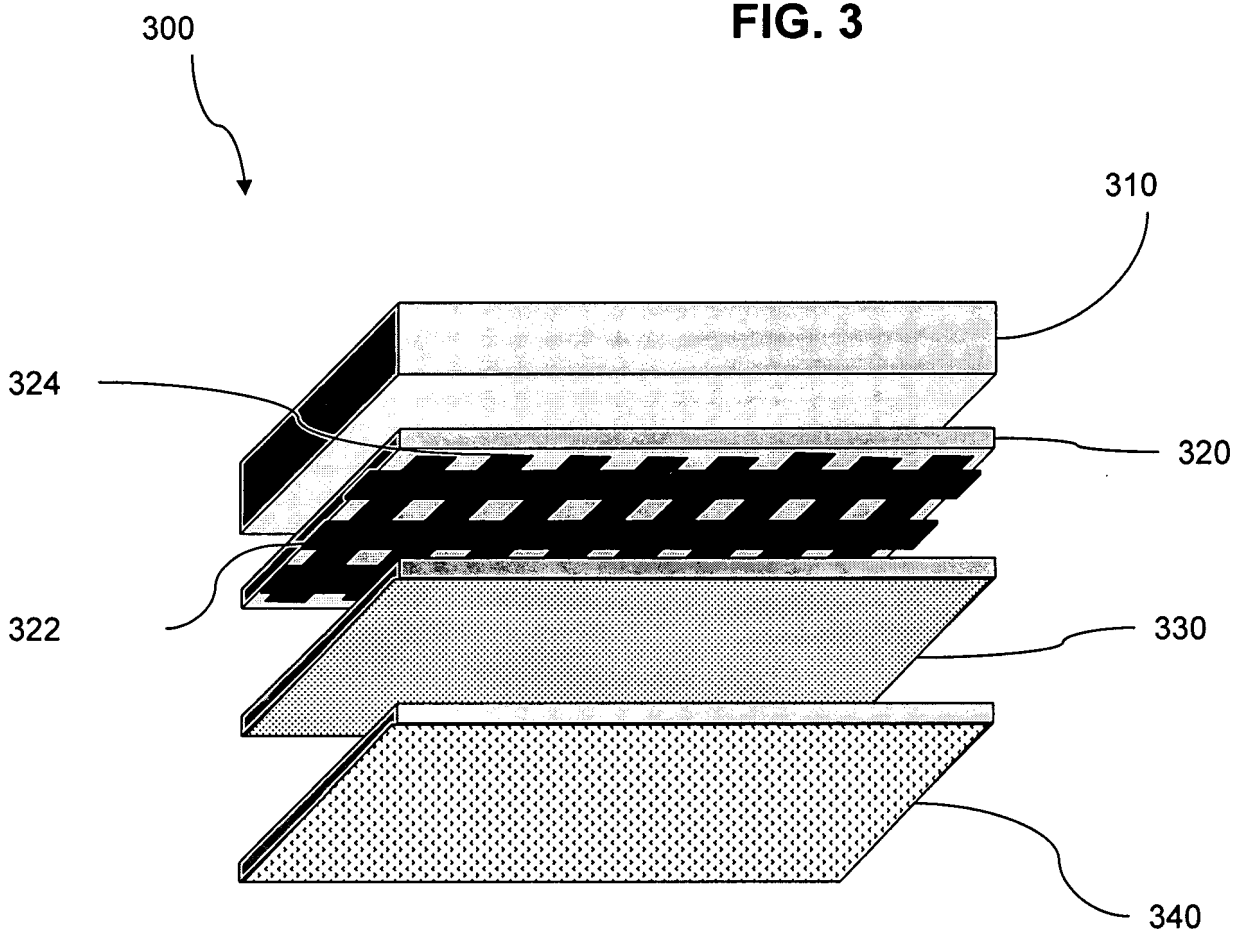
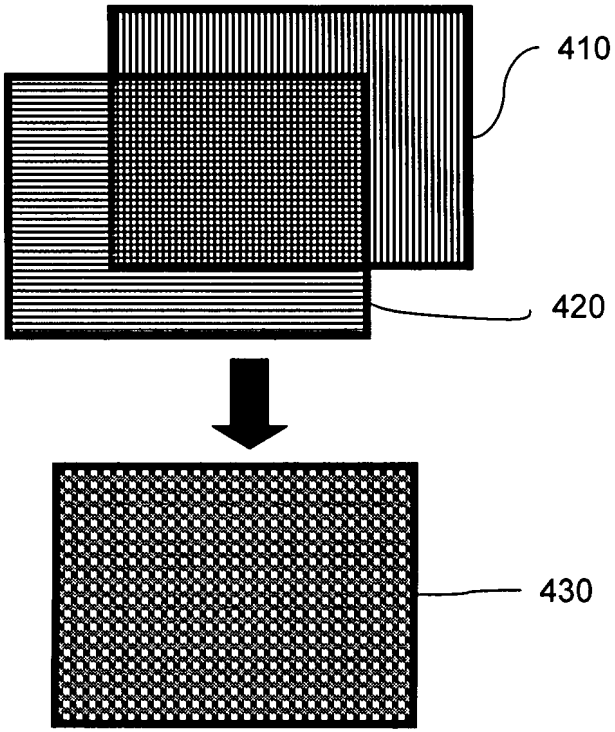
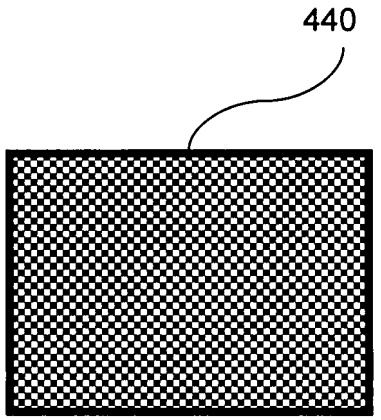


FIG. 4

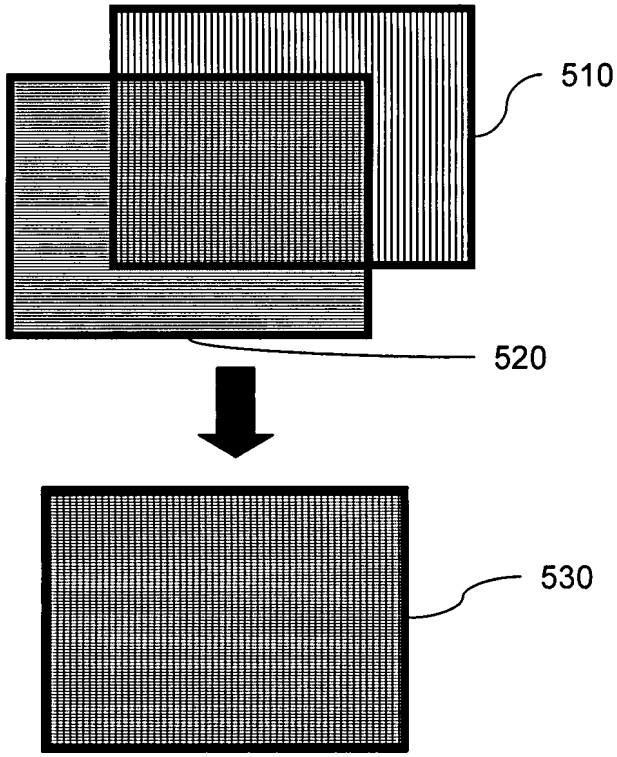


Independent overlapping
X and Y electrode fields

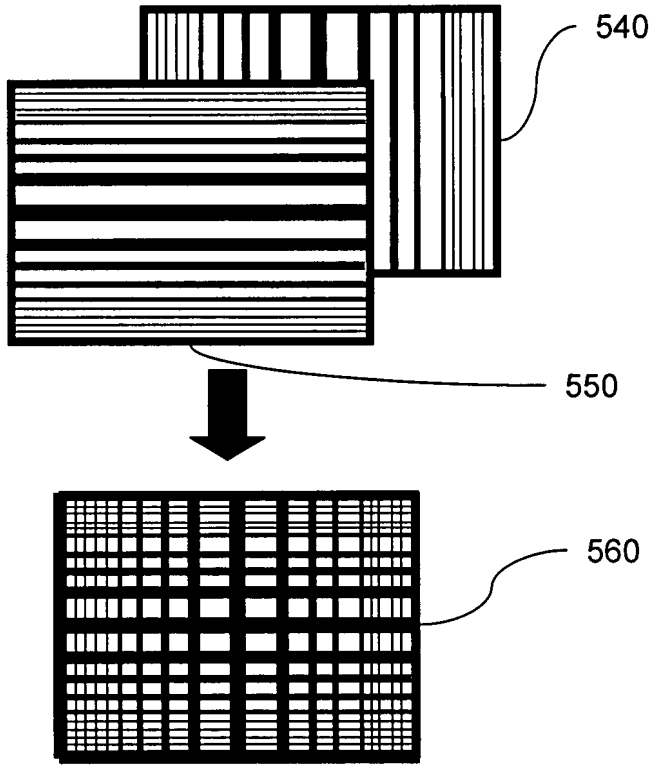


2 dimensional electrode pixel array
with overlapping X and Y data

FIG. 5



X and Y electrode fields
Of different electrode densities
Supports high density corrections
in X vs. Y directions



X and Y electrode fields
Of uneven electrode width or spacing
Supports spatially non-linear corrections

FIG. 6

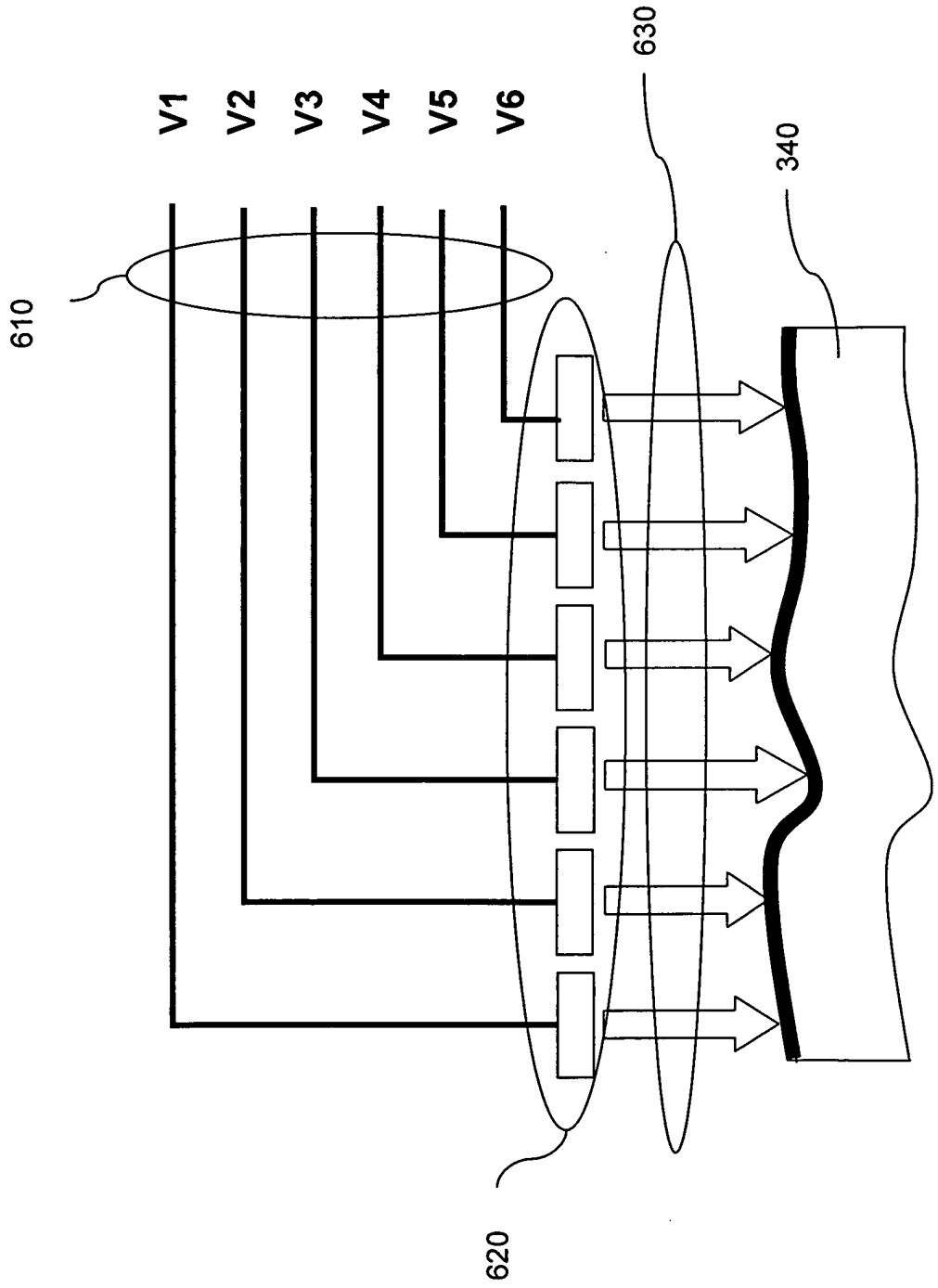


FIG. 7A

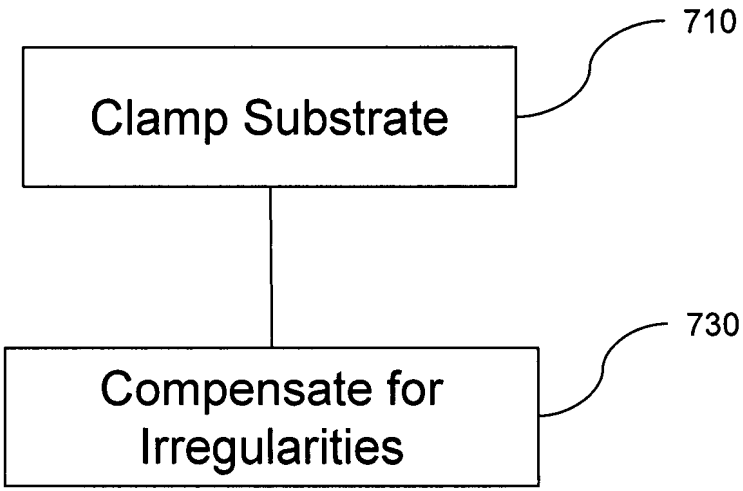
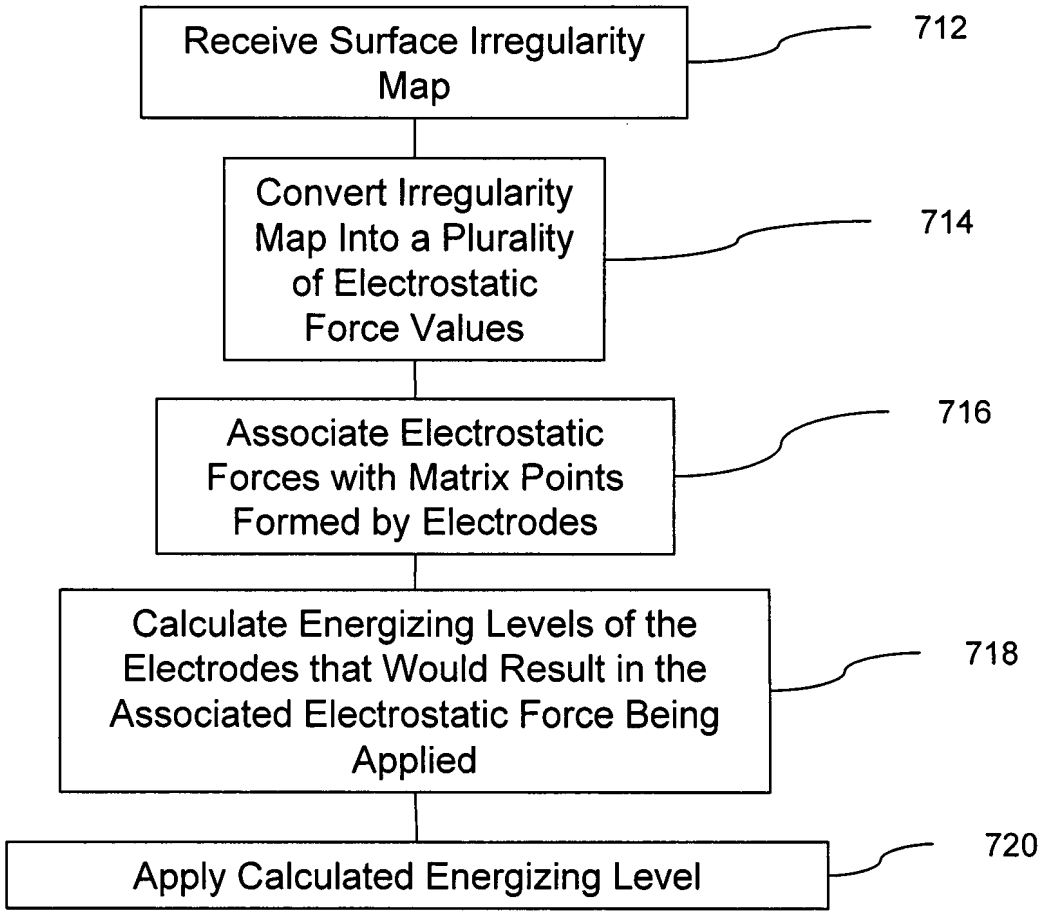


FIG. 7B


Receive Surface Irregularity Map
712
Convert Irregularity Map Into a Plurality of Electrostatic Force Values
714
Associate Electrostatic Forces with Matrix Points Formed by Electrodes
716
Calculate Energizing Levels of the Electrodes that Would Result in the Associated Electrostatic Force Being Applied
718
Apply Calculated Energizing Level
720

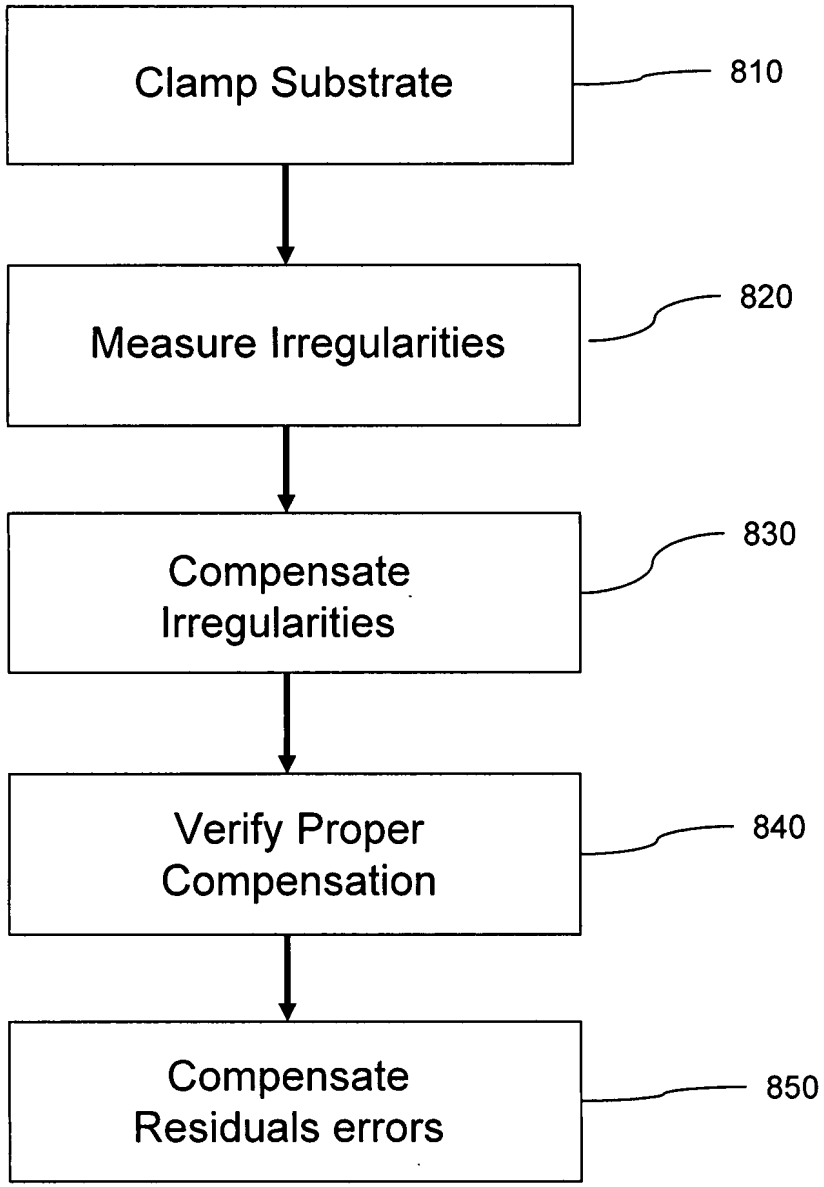

Clamp Substrate
810
Measure Irregularities
820
Compensate Irregularities
830
Verify Proper Compensation
840
Compensate Residuals errors
850

FIG. 8A

FIG. 8B

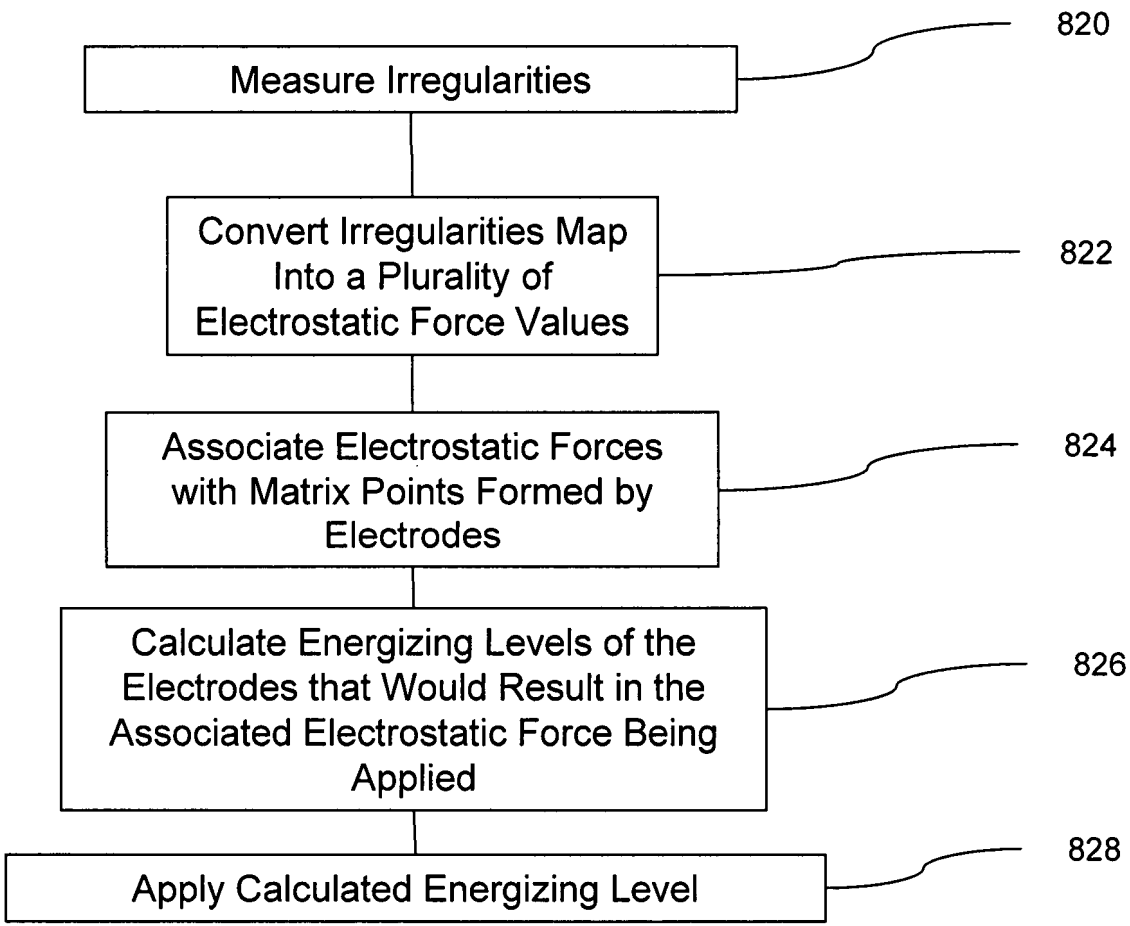

Measure Irregularities
820
Convert Irregularities Map Into a Plurality of Electrostatic Force Values
822
Associate Electrostatic Forces with Matrix Points Formed by Electrodes
824
Calculate Energizing Levels of the Electrodes that Would Result in the Associated Electrostatic Force Being Applied
826
Apply Calculated Energizing Level
828

FIG. 9A

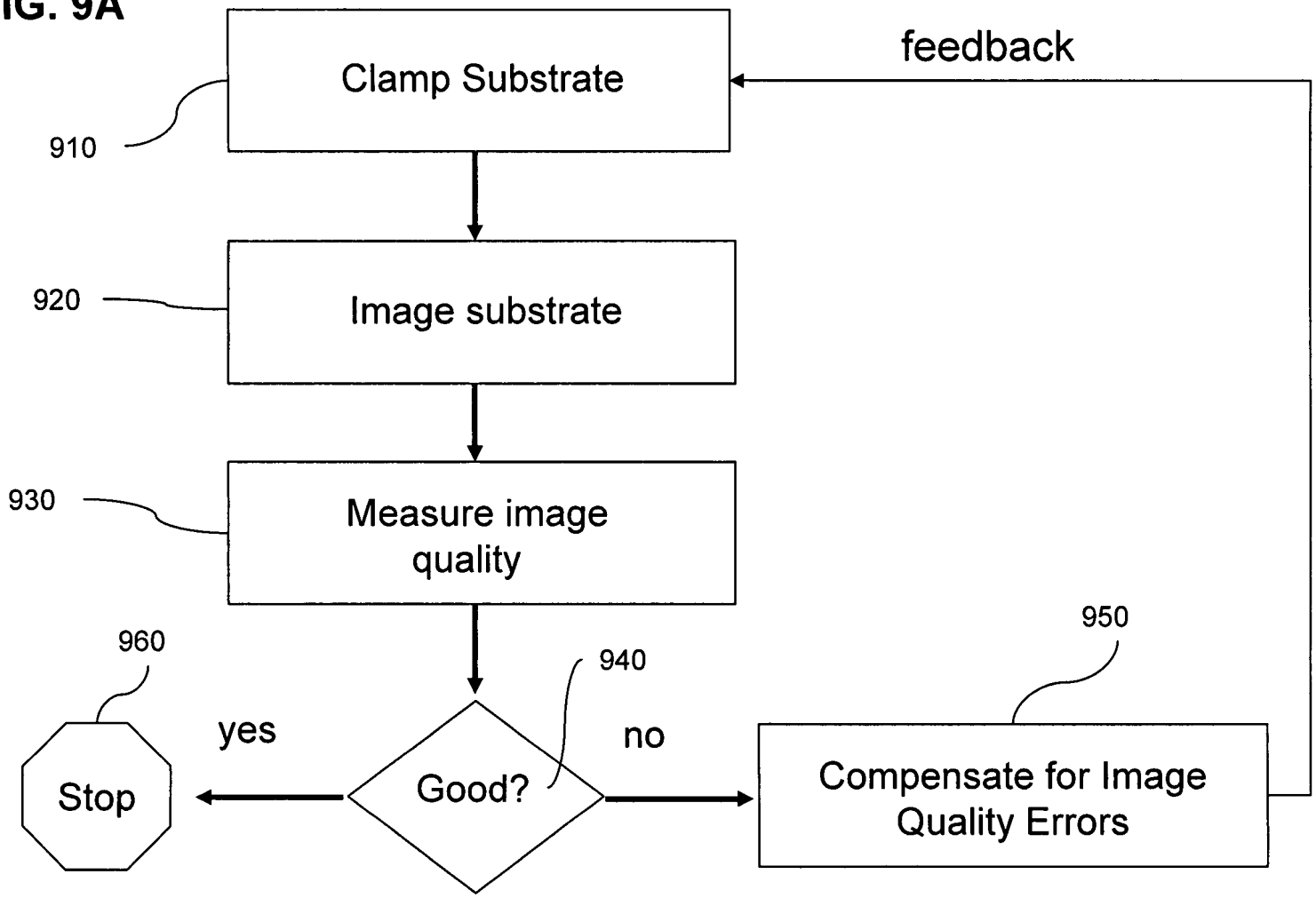

Clamp Substrate
910
feedback
Image substrate
920
Measure image quality
930
960
940
950
yes
no
Stop
Good?
Compensate for Image Quality Errors

FIG. 9B

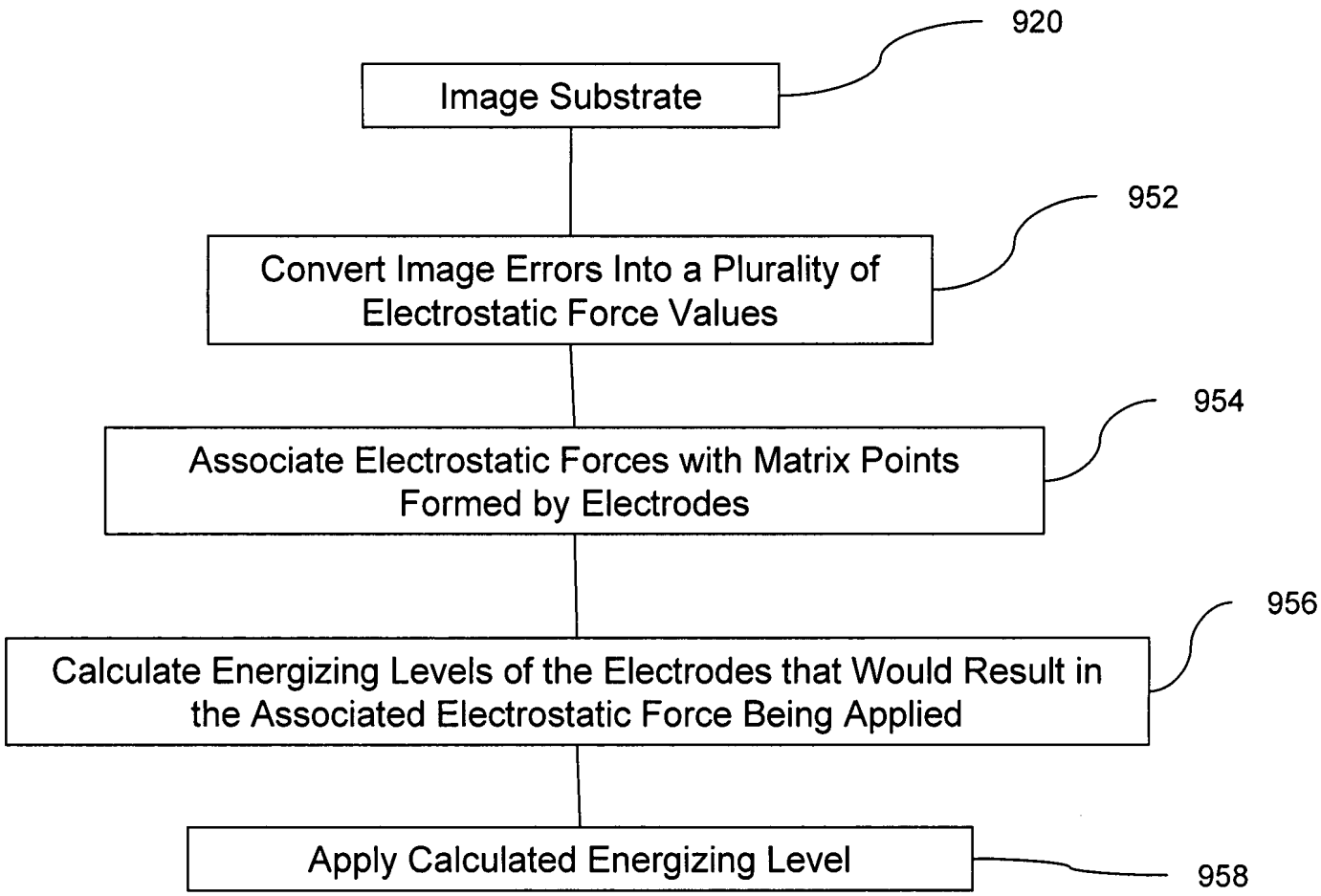
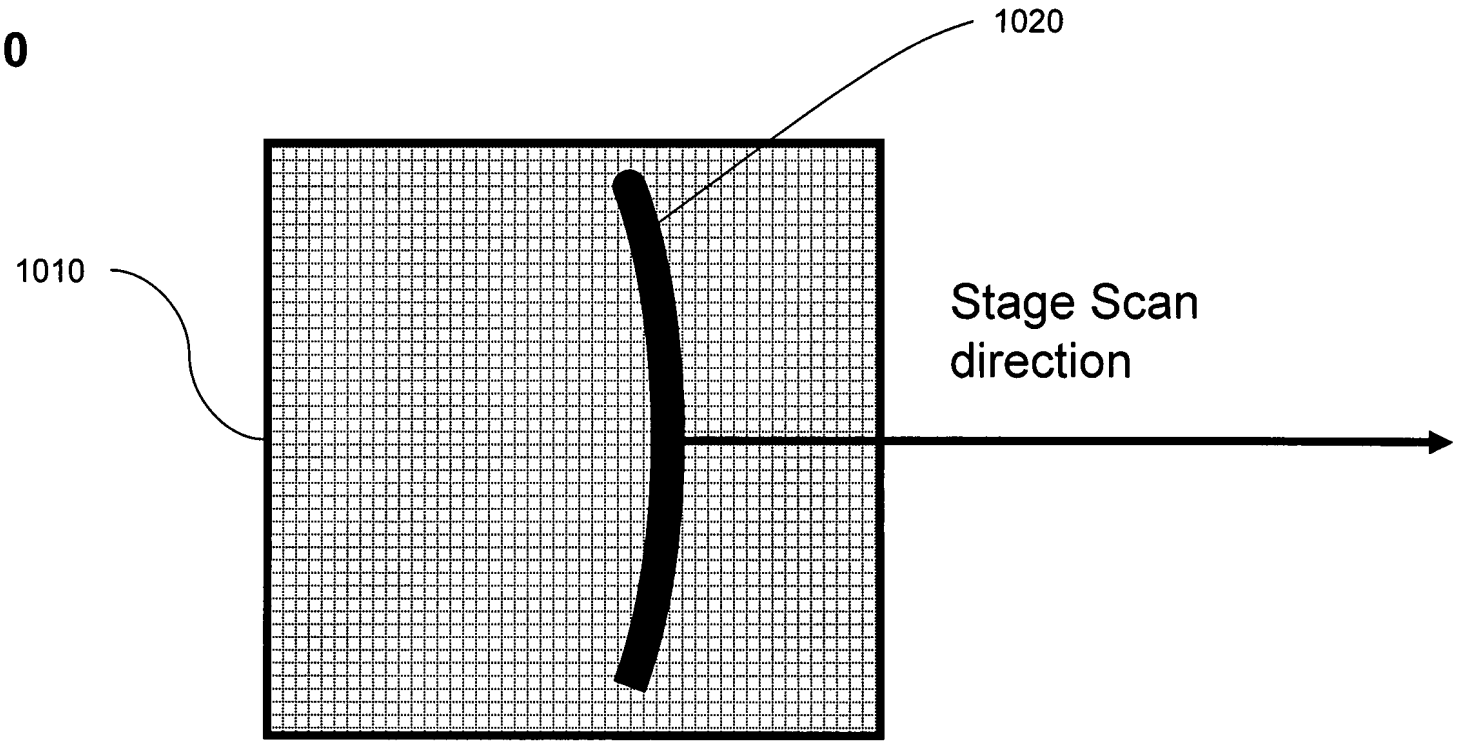

920
Image Substrate
952
Convert Image Errors Into a Plurality of Electrostatic Force Values
954
Associate Electrostatic Forces with Matrix Points Formed by Electrodes
956
Calculate Energizing Levels of the Electrodes that Would Result in the Associated Electrostatic Force Being Applied
Apply Calculated Energizing Level
958

FIG. 10



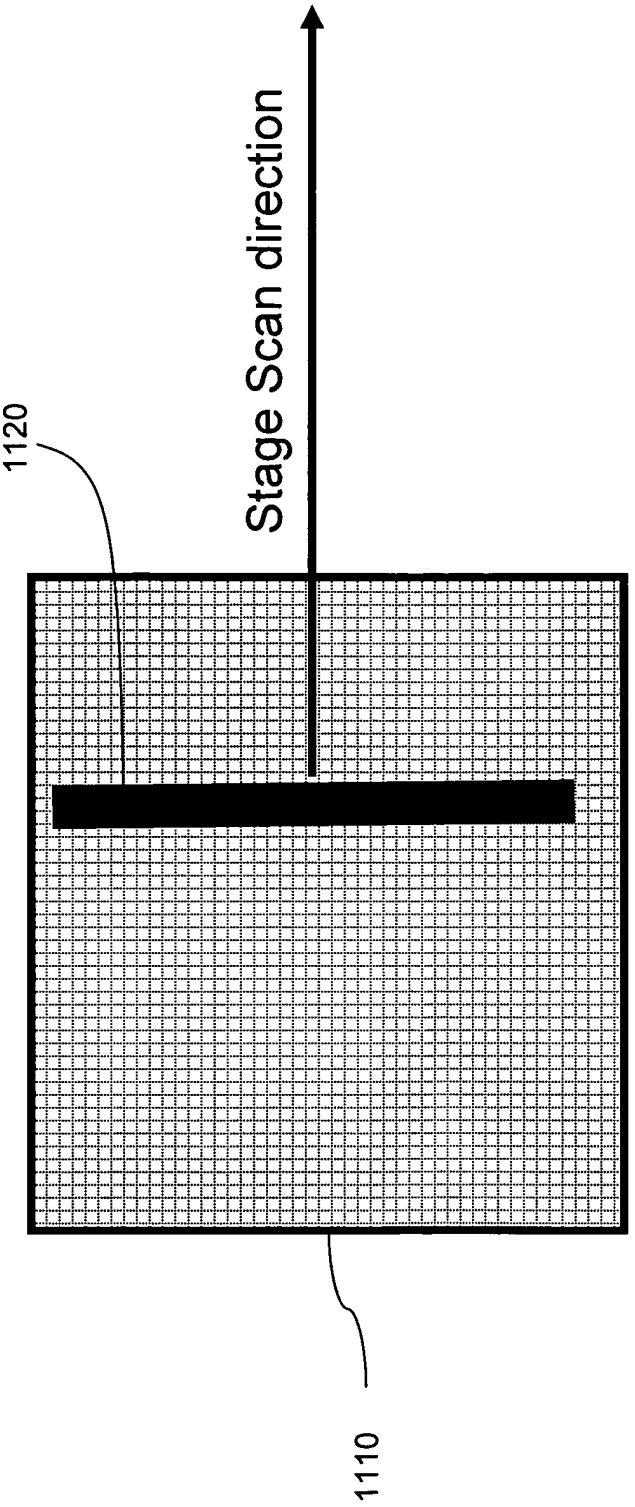
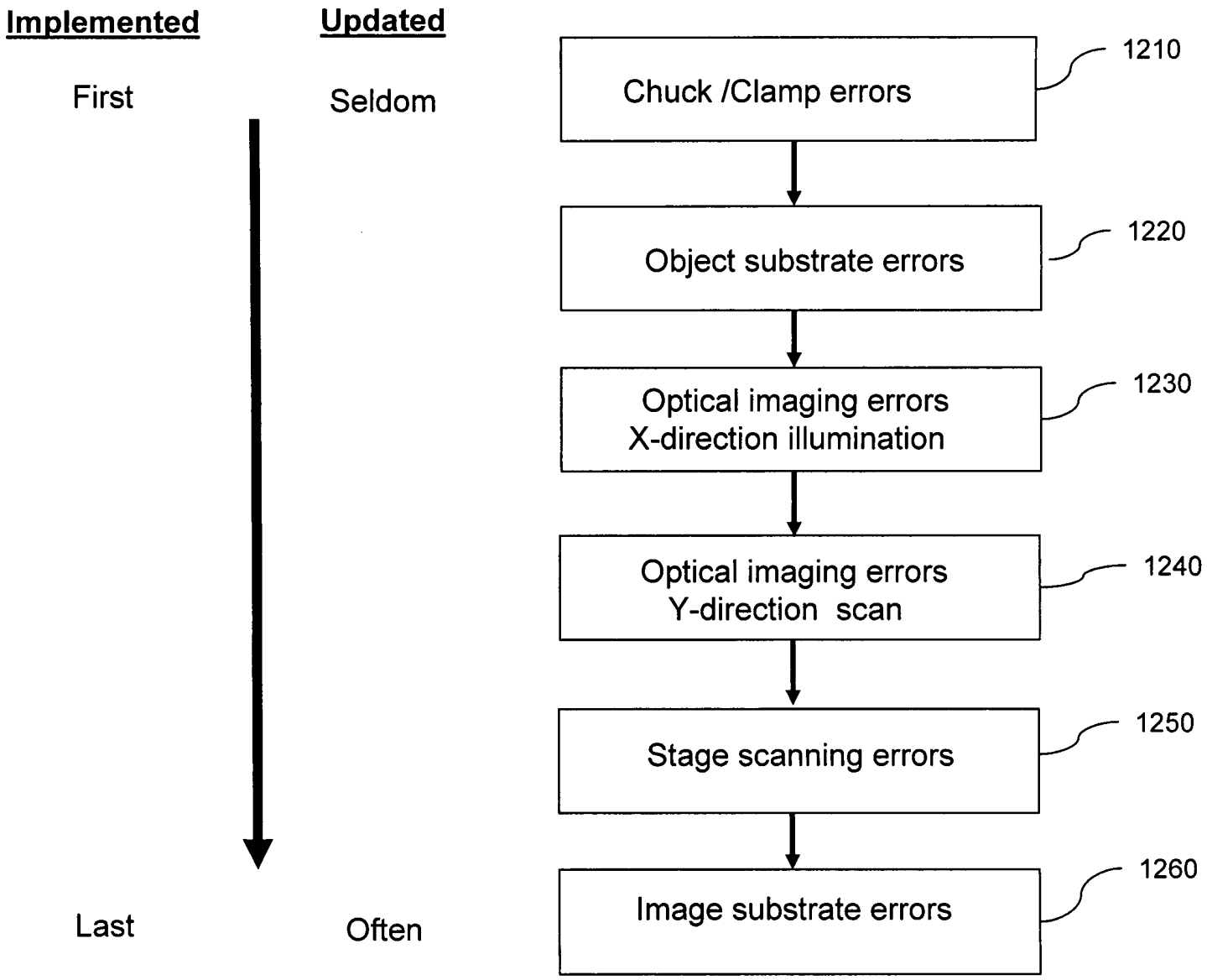


FIG. 11

FIG. 12


Implemented
Updated
First
Seldom
Last
Often
Chuck /Clamp errors
1210
Object substrate errors
1220
Optical imaging errors
X-direction illumination
1230
Optical imaging errors
Y-direction scan
1240
Stage scanning errors
1250
Image substrate errors
1260